

Causes of the British disease

Economists are once more glooming about the British economy, but looking for remedies in the wrong places.

JUST over a decade ago, the then Conservative British Government under Mr Edward Heath took fright that the unemployment rolls appeared to be growing inexorably and an inflationary budget was hastily put together out of season. There was hardly time to tell whether its intended effect — the creation of jobs — would have become reality, for the Heath Government was voted out of office after a bruising confrontation with the British coalminers' union. Now, with another Conservative Government in office, there is again a miners' strike (not fully effective), the pound sterling is trading more cheaply against other currencies than ever before and, this time, unemployment is twice what it was when Mr Heath took fright. Inevitably, people wring their hands in anxiety, even despair. What has gone wrong? And what can be done to put things right?

Most answers to these questions are political, usually in the partisan sense, and that, in the long run, is the language in which they will have to be answered. But there is also an sense in which the problem of the British economy is a model, not an especially uplifting model, for the way in which economic systems should be managed in times of rapid technological change. The curious feature of the British example is that it has been fully analysed and thoroughly discussed for at least the past forty years (since the end of the Second World War), and that even now, nobody is quite sure of what should be done. The government of the past five years believes that sound money is an essential part of any solution (which is probably true), and recently has taken to saying that a more flexible job market would also help (which is probably also true, but only up to a point). The chief opposition party, on the other hand, says that the chief need is to create more jobs, and is plainly envious of the way in which deficit financing of the Reagan Government's operations seems to have gone hand-in-hand with a revival of economic activity in the United States. The obvious difficulty is that the objectives at which the political parties have set their sights may be parts of a solution to the problems that afflict the British economy, but cannot be the whole of one.

Changes

That the post-war decades would be times of rapid change was apparent from the outset. That they would also be decades of rapid social change was also obvious, and was foreseen. The agenda for most speculations about what may happen to Britain was set in the five hectic years to 1950, a period of unprecedented and unmatched change. While voluntarily giving the notion of being the hub of an empire, the British embarked on two enlightened schemes — universal high-school education and a social security system and a health service to go with it — embodying the principle that bad luck (including ill-health) should not overwhelmingly determine the conditions in which people lived and died. During the same five years, several major industries (railways, coal, electricity generation and supply) were nationalized. (Steel and water came later, telecommunications were an outgrowth of Rowland Hill's Victorian Post Office and will be half-turned over to the private sector only next month.) It tends now to be forgotten, even in Britain, that from this earliest post-war period, government committees were repeatedly forecasting desperate shortages of trained technical people, and pleading that something should be done about the scale as well as

the quality of technical education.

In retrospect, this heady period of social and administrative change was lived through with too little calculation of what the costs might be. Throughout the 1950s, while economic growth picked up, taxation grew even faster, with the result that a succession of Conservative governments presided over the personal impoverishment of most of those who had voted them into power. This was nevertheless a period of great optimism about the way in which technology would soon make Britain prosperous again — nuclear power would be dirt cheap, civilian jet airliners would fill the skies and there would even be thermo-nuclear power for succeeding decades to enjoy. The truth became plain only in the winter of 1962–63, when much of Britain went without electricity for weeks on end because the weather was appalling and the demand could not be met. There had already been the Italian miracle to remind the British how easily pride of place in some economic league table can be lost by erosion at the rate of one or two per cent a year. In the circumstances, it is only natural that the Wilson Government of 1964 should have promised prosperity by stimulated technological revolution — and that it should have failed because the money supply ran away from it.

Mistakes

Throughout this now long period, two categories of mistakes have been consistently perpetuated. First, in spite of the lip-service paid by governments of all colours to the importance of technical education as a basis for the prosperity of the modern state, too little has been done to turn the dictum into a reality. Qualitatively, the system has hardly changed in half a century. Young people are still required to choose at high-school the directions in which they will follow intellectual pursuits, with the consequence that many of the most able people follow careers which are only peripheral to present needs, while those who take the technical route have a lopsided education. Now, half a century later, there are signs that this may change. Quantitatively, however, there is great confusion. The present happens to be a time when there are obvious shortages of technical skills, and of general engineering knowledge, but there have as often been times when British governments and their advisers have been willing to leave what they considered well alone. In the circumstances, it is unremarkable that those technical people who work in industry are badly rewarded and undervalued. The common wry comment, that Britain is skilled at invention but poor at exploitation, is too often considered an entirely unconnected circumstance.

The other consistent mistake is the assumption, to which all governments are prone, that there are some features of the working of an industrial economy that are best decided by civil servants, not by those who earn their livings in industry or even by market forces. The most memorable follies of this kind date from the 1960s, when the Wilson Government embarked on schemes for cementing mergers between successful and less successful companies when it would have been cheaper, and simpler, to let the latter go to the wall. The curious circumstance is that similar transgressions of good sense occur even under the present government, which is almost un-British in its belief that business failure is merely the other side of the coin of success. Only two



weeks ago, the Bank of England saved from bankruptcy a bank that should have been allowed to go to the wall. The same government is doing everything it can to encourage the growth of a few high-technology sectors without recognizing that they are unlikely to succeed unless there is a climate in which intermediate technology can also prosper. But as if to show its even-handedness, the government also goes to great trouble to ensure that its chosen sunrise industries fall flat on their faces needlessly. Thus it is that the two most experienced operators, Rediffusion and Visionhire, last week pulled out of the nascent cable industry, the brave new industry of 1982, because the terms of operating licences have been made unduly onerous.

The consequences of all this are evident. The free market in manufactured goods and value-added services is both hamstrung and feather-bedded. Why should the labour market be singled out for exceptional efficiency, with all the political trouble (and inequity) that would cause? But the civil service, acting as proxy entrepreneur, makes mistakes whose cost is never counted, but which can be measured by the collective impoverishment. But these are the circumstances in which, in a mobile world, people who think they can better themselves elsewhere are up and off. The cost to Britain of the shiploads of those who have left for good, convinced that Britain has nothing much to offer, is incalculable.

It is in the circumstances no wonder that the word has got about that Britain is not serious about its talk of joining the modern world. What sense can it make that a government seeking to sponsor a new industry should cripple it with artificial conditions that prevent it from being viable? Or that it should delegate decisions such as whether there should be another nuclear power station in Britain, not to the nationalized industry that would build and operate the plant, but to a one-man tribunal (with two advisers) sitting daily in Suffolk for the past two years? Would it not be better that the government should tackle the problems that nobody else has the power to tackle, such as the reform of public education? And should it not from time to time make speeches that give some hint that it is not entirely overwhelmed by the despondency about the future which, for want of evidence to the contrary, must be the general conviction. □

Argument too formal

The US National Academy has a new journal on public policy, which deserves to succeed.

AFTER more than a decade of introspection, the National Academy of Sciences in Washington has published the first issue of its journal concerned with the general implications of its members' professional work. *Issues in Science and Technology* is as handsome a publication as would be expected, given both the sources (the National Academy of Engineering and the Institute of Medicine are also partners in the venture) but also the ambition — to provide a regular (quarterly) commentary on important issues and related public policies that will compare in influence with publications such as *Foreign Affairs*. The Fall 1984 issue deals with some of the issues certain to be recurrent themes in the years ahead — ballistic missile defence, the cost of health care and the regulatory impediments to the application of technical innovation (specifically, the introduction of air bags as safety devices in impacting automobiles). At \$6.00, the first issue is a good read, or at least an interesting one.

What will follow next is, naturally, not necessarily determined by this first advertisement of what the academies have in mind. Next time, for example, there will be no need to reprint the article by Daniel Yankelovich, the social pollster, retailing a simple account of why public disenchantment with science and technology has been growing since the 1960s. (The influence of articles on this same theme has not, unfortunately, been recorded.) Arms control is, however, certain to be a recurrent theme, and the first issue includes a pair of opposing articles, by Dr George A. Keyworth and Dr Sydney Drell, on star wars and related matters. Most people will find this a valuable summary of

influential opinions on the subject.

So haven't the academics been brave, venturing so far from academic ways? That is what the blurbs suggest. The trouble is that neatly counterbalanced articles are less a way of helping to form opinion than to inform it. If *Issues* is to win the influence it seeks, it will at some stage have to climb down off this fence, not so as to be partisan but to avoid the appearance of seeking equal time within every pair of covers! And it will have to carry regular book reviews, not just thumbnail sketches of them. But the enterprise is brave; we shall all be better off if it succeeds. □

Truth will out (if late)

The departure from the United States of a once-German rocket engineer is a chilling lesson.

THE surprising announcement last week that Arthur Rudolph, who designed the Saturn V rocket, had left the United States and given up his US citizenship to avoid deportation as a Nazi war criminal should be enough to convince the most dedicated pragmatists of the moral contradictions in the recruitment of Nazi scientists at the close of the Second World War. More than a hundred Nazi scientists and technicians were then whisked to the United States in an effort to exploit Germany's commanding expertise in rocketry at that time — and, perhaps of greater importance to the US military commanders who directed the operation, to prevent Nazi experts from falling into the hands of the Soviets, as many nonetheless did.

The United States was plainly eager to overlook the obvious fact that these scientists had, with no apparent moral qualms, fully supported and enthusiastically worked for the Nazi regime; scientists are always called upon to build weapons for their country during wartime, the easy rationalization went, and these scientists just happened to be in the wrong country. It was not their fault; they were just following orders. And, mass memory being what it is, few in the United States seem to have been genuinely troubled that their space programme was partly run by the men who built the V-2. A rare dissent was registered in the 1960s by satirical songwriter Tom Lehrer, who summed up the amorality of the rocket scientists with his biting irony, ascribing to Wernher von Braun the opinion that where the rockets came down was "not my department".

The Rudolph case offers a reminder that really should not be needed that Nazi Germany was not just another country at war. Rudolph's job as director for production of the V-2 made him not only a collaborator with the German war effort, but with the most enormous of the Nazi horrors as well. The workers in the underground factory that produced the V-2 were slave labourers and concentration camp inmates. Of the 60,000 prisoners who passed through the Dora-Nordhausen concentration camp adjacent to the factory, one-third died. The Justice Department now says that Rudolph "participated in the persecution" of these labourers, who were worked 12 hours a day, starved, forced to sleep on bare rock in the tunnels and beaten.

The United States Government did not require 40 years to discover these facts. Rudolph was interrogated by US Army investigators in 1947, on that occasion giving contradictory answers to questions about treatment of prisoners, ultimately acknowledging that he had witnessed the execution of 12 prisoners accused of sabotage. But only the establishment in 1979 of a special office within the Justice Department, charged with investigating suspected war criminals in the United States, seems to have led to the recent action against Rudolph.

There is another reminder in this story. As much as researchers (and, it seems particularly, university administrators) like to maintain that science is not to be judged by its "end use", there are exceptions. Scientific research is a neutral, amoral enterprise only in a free society, one with a democracy capable of exercising social judgement over the propriety of end uses. In the absence of such social judgement, scientists have an obligation to exercise a personal moral judgement, to think through the consequences of their actions. □

British research council

Science council looks for projects to cut

THE British Science and Engineering Research Council (SERC) decided at its council meeting last week that it must withdraw from some of the research fields it supports. The council has given itself until February to decide how the pattern of its activities should be reshaped. "Major acts of surgery" are on the cards, according to Professor John Kingman, chairman of the council, speaking after the council meeting.

By Kingman's account, the council is now at a "turning point" in its affairs. Since this time last year, when the British Government refused the request from the Advisory Board for the Research Councils (ABRC) to provide more money for research, Kingman says the council has seen trouble coming. While the total research budget has been formally indexed against inflation, Kingman says that for various reasons it is being eroded by several per cent a year.

Among the council's immediate difficulties is a demand by ABRC that it should contribute £7 million towards the cost of reorganization at the Agricultural and Food Research Council, which is having to close a number of institutes under financial pressure. Kingman says he is "bitterly disappointed" that no extra help has been provided on this account. But the council has also been further hit by a decline in the value of sterling in the past few months, and by improvements of some university salaries (to which many post-doctoral stipends are tied).

Kingman says that with this project, the choice is between uniform cuts across the board and "more radical decisions" that will entail pulling out of some activities. For much of the past year, a group of council members and officials has been considering what steps of this kind might be taken, and what the financial savings would be. One conclusion is that a decision to shut down an institution or to abandon a research programme will yield full savings only over a period of five years. This is why, Kingman says, the impending cuts are meant to increase flexibility only in the 1990s.

For the time being, nobody is prepared to guess what will be cut. At pains to forestall the rumour mills, Kingman last week emphasized that the period between now and February will be used for a re-examination of the research programmes supported by the council's four spending boards (responsible for nuclear and high-energy physics, astronomy and space, engineering and general science). Each board will be expected by February to have "gone back to square one", and to present

a new case for its claim on council funds. Kingman says that at last week's council meeting, he asked for and was given an assurance by the four board chairmen that they accept the need for a radical reappraisal.

The task of listing projects and institutions that might be abandoned, and of working out the savings that may result, will fall on SERC officials overseen by a steering group appointed by the council. It seems to be accepted that there will be no time for full consultation with those affected before decisions must be made in February, although Kingman says that there will still then be a need for individual boards to choose between options that will have been presented to them.

This tight timetable, which may be changed, is conditioned by the need that the council should disclose to ABRC its spending plans by next April, at the beginning of the next financial year. SERC hopes that the Kendrew committee's recommendation on continued membership of CERN (the European Organization for Nuclear Research) will be available by February, but "if not we shall have to do our own study". Pulling out of high-energy physics would "be an easy option" but could not give the council the flexibility it needs — it would merely "buy time" for perhaps five years.

Kingman is not hopeful that significant economies can arise from changed

relationships with other research councils. He considers that there should be a closer relationship between SERC and the Natural Environment Research Council, however, and that ABRC should give thought to British support for research in biology. Economies through European collaboration accrue only when partners have money to contribute to joint projects.

Although the British Government's allocation of funds for 1985-86 and thereafter could still be increased above the amount forecast last December, nobody seems to think it likely that there will be a last-minute change of heart. Kingman last week also complained that last year's agreement with the Treasury about compensation for the depreciation of sterling, which yielded £7 million out of a total shortfall of £10 million, would have to be negotiated all over again this year, perhaps this time unsuccessfully.

The council seems to be entirely serious in its intentions. Professor Kingman last week quoted ABRC evidence to a House of Commons committee in support of his estimate that SERC would be £70 million short by the end of the decade (in a budget then estimated at £280 million a year). He said nevertheless that he welcomed the strategy document from the University Grants Committee, promising increased selectivity if "the other half of the dual-support system were properly managed".

On the question whether the council could have made its decisions sooner, Kingman said the investigation of what might be done had started a year ago, but that council would then probably have been unable to accept the need for the tough decisions it had now, in worsened circumstances, recognized to be necessary.

John Maddox

Medical shoestring pared down

A FURTHER drawing-in of horns in support for academic research in Britain is forecast in a letter from the secretary of the UK Medical Research Council (MRC), Sir James Gowans, to vice-chancellors of British universities. The letter, published last week, says that the council has no choice but to reduce the funds available for meeting project grant applications in the coming financial year by 7.5 per cent. Programme grants, used for supporting longer term projects, sometimes of a more open-ended character, are to be reduced by 25 per cent, while the funds available for paying stipends to postgraduate students and trainees will be reduced by no less than 30 per cent.

The council's letter, an unusual advertisement of its financial problems, explains that part of the reason for the substantial cut in prospect is the contribution, of about £2 million, that MRC will be required to make to the cost of reorganizing the Agricultural and Food Research Council. But it seems that the extra cost of university

salaries, which affect the council's budget for expenditure already agreed by increasing automatically the salaries of research fellows, will cost the council more than £1 million next year.

As might be expected, the council's letter says that these further economies are made with regret. It also says that if there is some alleviation in the financial position, perhaps because the government accepts its case that its budget should be increased, it may be possible to increase the sums available for next year.

The surprise is that the council should have gone so far as to say that its case for extra money has been urged on the government, in the persons of the Prime Minister, Mrs Margaret Thatcher, and the Secretary of State for Education and Science, Sir Keith Joseph, by the secretary of MRC and its chairman, Lord Jellicoe. From the evidence of the latter now published, the meetings were particularly joyless even as these occasions go.

John Maddox

US Congress sweeps up remaining bills

ASATs, agriculture and antitrust

Washington

AMONG the last-minute business that the US Congress completed before adjourning to hit the campaign trail was the resolution of three long-standing issues in science policy and arms control:

Antisatellite weapons

MOVED to act by the absence of any discernible leadership on this issue from the White House, Congress imposed its own moratorium on testing of the new US antisatellite device, a small nonexplosive rocket launched from an F-15 fighter. The Air Force has so far conducted only one test of the weapon, which on that occasion did not carry a warhead. (The warhead, which has been described as a "smart rock", technically the Miniature Homing Vehicle, manoeuvres into the target satellite and disables it simply by ramming it.) The congressional ban is on testing against a live target — seen as the critical step in proving the weapon.

Under the compromise finally worked out between the House of Representatives, which had wanted a one-year ban, and the Senate, which favoured only a requirement that the President should convey to Congress certain assurances that the testing was necessary, no tests are allowed before 1 March of next year. After that, and before tests can begin, the President will have to certify that tests are necessary to prevent harm to national security, that they are consistent with the anti-ballistic missile (ABM) treaty and will not irreversibly harm chances for negotiation of an antisatellite weapons (ASAT) limitation treaty, and that an effort is being made in good faith to negotiate such limitations with the Soviet Union.

The law also provides for a 15-day waiting period after the President's certifications, during which time Congress could presumably vote to extend the moratorium — although any such vote would be subject to the President's veto. And no more than three tests against a target will in any case be permitted during the 1985 fiscal year, which ends on 30 September 1985.

Technical or political reasons, or a combination of the two, have delayed the second planned test, which has been expected for some time. In this test, the weapon, with warhead, will be shot at an imaginary target in space. Rumour has it that this test will occur within the next few weeks.

The limitations passed by Congress are a small but nonetheless important step — it is the first time that Congress has legislated on arms control. Representative George Brown (Democrat, California), a principal sponsor of the moratorium, said the measure "provides a *de facto* arms control

agreement" that buys time "to get negotiations back on track".

No limitations have, however, been imposed on the US Air Force's budget for procuring the weapons. Congress approved \$74 million for that purpose. The research and development budget is \$133 million.

Agriculture

THE catch-all appropriations bill passed by Congress, which will keep the government operating until the end of the 1985 fiscal year, includes a generous budget for the habitually impoverished competitive grants programme for agricultural research. Created in 1977 as an alternative to the formula-funding system — long criticized for its absence of peer review and its exclusion of non-land-grant colleges — the programme has been held to a mere \$17 million by those in Congress — notably Representative Jamie Whitten (Democrat, Mississippi) — who saw it as a threat to business as usual.

This year, Congress agreed to \$46 million, close to the \$50 million requested by the administration. Efforts by Whitten to keep up the appearance of a budget increase while undermining its substance were, however, modestly successful. Within the \$46 million is \$7.5 million earmarked by Whitten for specific programmes, apparently transferred wholesale from "special grants" — congressionally-mandated research that the US Department of Agriculture has never cared for and which are finally being eliminated this year. Thus while the general area of plant sciences was voted an increase of \$1.5 million from last year's \$15 million, \$1.7 million was set aside for special projects in soybeans, acid precipitation and alcohol fuels. A new area of "pest sciences" turns out to be devoted completely to three special projects, with \$1 million apiece: studies of boll weevil, pine bark beetle and gypsy moth. And of the \$4.5 million appropriated for a new area of animal sciences, \$3 million is committed to special projects.

Real gains were, however, made in the creation of a new \$20 million biotechnology area, with no strings attached. The human nutrition area will receive \$2 million, as before.

The director of the competitive grants programme, Ed Kendrick, says that although the incorporation of the special projects into the programme will take some careful planning, all the money will be awarded on a competitive basis.

Most attention now will turn to the budget for the next fiscal year, due next February. The first substantial increase in competitive grants for several years is bound to whet the appetites of their

advocates.

Requests for proposals in the new programme area will be announced in mid-November.

Antitrust exemption

HIGH-technology companies in the United States have been pressing for an exemption from antitrust laws that would allow them to collaborate on research without fear of landing in court. The inevitable comparisons with Japan were heard over and over again at congressional hearings least spring. Legislation signed by the President on 11 October confers a degree of protection upon such joint ventures, although it stops far short of the blanket exemption that industry had hoped for.

Under the principal antitrust statutes, a company engaged in anticompetitive practices can be sued by a competitor for three times the competitor's actual monetary damages. The bill just enacted limits that to actual damages in the case of joint ventures in research and development, so long as the companies entering into such collaborations disclose their activities in advance to the federal government.

The government will print a notice in the *Federal Register* listing the parties and a general description of their activities; although more specific information will have to be supplied to the government, those details will be protected against release to the public.

The theory behind the new law is that although joint ventures in research and development have never been illegal *per se*, the threat of triple damages has made companies overly cautious. And, in turn, companies involved in such ventures are likely to toe the line if they know that the Justice Department is keeping an eye on them.

The new law provides another protection for joint research and development ventures by allowing courts to award attorneys' fees to defendants who are the victim of frivolous lawsuits.

Whether the law will be the tonic to US industry that its high-technology advocates claim is questionable. For one thing, joint research and development ventures have almost never been the target of antitrust actions anyway. In the past 25 years, only four cases have been brought; and none of these questioned the joint collaboration *per se*.

The best known case was brought by the Justice Department against the major US auto-makers, which had collaborated on pollution control research (which was not illegal) but then allegedly conspired to delay the introduction of that technology. In another famous case, a photographic company sued over a joint research venture that Kodak had entered into with virtually all other photographic companies, excluding only the plaintiff in the case.

Stephen Budiansky

Carlsberg Foundation

Research rides on beer sales

SOME countries have natural resources; so does Denmark. It has beer. And Danish beer benefits science for, strangely enough, the United Carlsberg and Tuborg breweries are run by five professors for the profit of research. And since conservative Danes drink nothing but Carlsberg and Tuborg, and since the breweries have been investing effectively in breweries abroad (Britons now drink more Carlsberg than the Danes), the Carlsberg Foundation has been doing well.

In fact, the Carlsberg Foundation for Research has increased its spending by 20–25 per cent a year for the past four years, and it now disburses annually as much as the Danish natural sciences research council. A fair fraction of the foundation's money goes to the humanities, but that still leaves a fine sum for science, from astronomy to microbiology. Yet, says foundation secretary Niels Petri, most of the recent increases have been taken up in "supporting unemployed scientists". And future growth is threatened by new tax laws.

Beer also pays for the Carlsberg Laboratory, supported directly by the breweries as a tax-break, which is free to range (in style) over much basic agricultural and molecular biology. In the past, the laboratory was responsible for the first cloning of a microorganism (E. Christian Hansen's pure yeast cultures), S.P.L. Sørensen's concept of pH, Linderstrøm-Lang's concepts of protein structure and function and — they say — Schmidt's discovery of eel migration and Winge's discovery of sexuality in yeast. More recently, the laboratory did less well, but now in quite extraordinary new premises there appears to be something of a reformation at the Carlsberg.

"Recruiting? We've never had to do any", says Carlsberg Laboratory director of physiology, Professor Carl von Wettstein. According to a Danish student, a scientist can get to work at the Carlsberg only "by invitation". The laboratory, however, has few permanent jobs. Of the 150 researchers at the laboratory at any one time, many will be postdoctoral people from abroad working on foreign money.

"But we get more results per kronor than any other institution in Denmark", claims von Wettstein. And the fact that he has to apply for outside money, from the European Communities for example, is proof that the laboratory is not over-generously supported, he says.

The only problem of working at the Carlsberg, say researchers there, is a strict requirement to publish in the house journal *Carlsberg Research Communications*, which has a circulation of only 400. But, says von Wettstein, the good work gets out anyway; the journal is critically refereed; it is not so affected by fashions as other journals; and it can report steps in major pro-

jects that take years to complete, as in the Carlsberg work on chlorophyll synthesis. "This was very fashionable in the 1960s, then everyone dropped it because it was too difficult. But we carried on and published in our journal."

In spite of acknowledged successes, some observers say the foundation could be more adventurous in the way it uses its funds. The five professors who direct it have considerable power to influence the directions of Danish science, but they usually walk in strict step with the research councils. Moreover, foreign beer sales are levelling off, and Petri expects the foundation's funds to level off next year, quite apart from possible tax changes aimed at a number of foundations somewhat less charitable than the Carlsberg.

Robert Walgate

Synchrotron radiation

THE French and West German governments have agreed to place the European Synchrotron Radiation Source (ESRS) at Grenoble, in the foothills of the French Alps, and not at Strasbourg as previously predicted by French government and scientific sources. This will put ESRS alongside the extremely successful high neutron flux beam reactor of the Institut Laue Langevin, and the announcement (last Friday) has been greeted with enthusiasm as making scientific sense.

Whether it makes political sense, however, is unclear. According to the announcement, ESRS will be a Franco-German facility; but the outline diagnosis of the synchrotron was done not just by France and Germany but also by the United Kingdom, Italy, Denmark, Sweden and Finland, two of which (Denmark and Italy) had put in firm site proposals.

Denmark has protested about apparent French hijacking of the project. All seven countries had been paying the costs of a study under the auspices of the European Science Foundation (ESF) to bring up ESRS, originally designed in 1979, to modern standards. The 300-page draft of the new design was sent to officials of the seven countries earlier this month, and a meeting of the ESRS intergovernmental programme committee (under ESF) is due to take place in Brussels tomorrow (Friday), ostensibly to discuss that design.

A French government spokesman on Monday was, however, unable to explain the relationship between the Franco-German ESRS decision and the ESF committee. Would ESRS be solely for the use of France and Germany? "France believes in creating a 'European space for research'" he said, quoting Prime Minister Laurent Fabius, "so ESRS will be open to all Europe".

Robert Walgate

Genetic manipulation

Agency tries out regulations

Washington

THE US Environmental Protection Agency (EPA) last week formally announced its interim policy on field tests of microbial pesticides (*Federal Register* 49, 40659–40661; 1984). Citing a "higher degree of uncertainty in predicting ecological impacts" of microbial as opposed to chemical pesticides, EPA will in future require 90 days' advance notice of field tests of "genetically altered or manipulated" organisms, or of those not indigenous to the test area. Hitherto, field tests of pesticides on plots smaller than 10 acres have been exempted from the notification requirement.

Industrial companies seem pleased with EPA's efforts to regulate in this area. The Monsanto Corporation has already given notice of its intention to carry out field experiments that will be controlled under the new policy; Robert Conken, director of registrations for the company, said the rules would satisfy the public's demand for regulation without impeding research.

As expected, EPA will not require a formal "Experimental Use Permit" for every test. Under the new policy, companies will, however, need to provide EPA with complete information about the proposed test, including details of the ecology and physiological tolerances of the test organism, as well as a complete description of the manipulations performed and their expected results. The agency will also require proposals for monitoring any spread of the microorganism and evaluating potential adverse effects, together with methods for disposal of exposed materials. The test will be allowed to proceed if EPA raises no objection in the 90-day notification period.

There were, however, some questions raised by industry about the scope of the new policy. Conken asked how a company should determine whether an organism is "non-indigenous" and so within the new guidelines: would an organism that occurs naturally elsewhere in the same state, for example, be counted as indigenous? There is similar uncertainty about the meaning of "genetically altered or manipulated" — the question being whether organisms obtained through artificial selection, for example, are included. But many of the questions are likely to be answered quite soon, when EPA produces a policy statement that will also cover releases of genetically-engineered or non-indigenous organisms other than pesticides. The agency plans to use the existing Toxic Substances Control Act for non-pesticides (see *Nature* 23 August, p.613). And EPA officials stress that no decisions in this area should be regarded as final: the agency is by its own admission feeling its way, and comments are invited on how the regulations should be improved.

Tim Beardsley

US space station

Anxieties abound in Europe

THE British Government is steeling itself for a decision on whether or not Britain should participate, at an estimated cost of £40 million a year, in the development of the US manned space station. But it can expect little direct financial help from British industry, which considers that investment in such a long-range project is a matter for the government. Such was the general feeling of participants at a symposium staged two weeks ago by the Department of Trade and Industry (DTI)



to sound out the views of industry and science.

For the British Government, part of the problem is that space spending will have to increase because of the demands of the European Space Agency (ESA). The agency's Horizon 2000 study earlier this year recommended an increase of the science budget by 7 per cent a year over five years. Britain now contributes £13 million a year, based on the Gross National Product. Failure to pay the proposed 40 per cent increase by 1990 could exclude Britain from a series of ambitious ESA projects, including a new X-ray observatory, a sub-millimetre orbiting telescope and a probe to return samples from a comet or asteroid.

The need for extra cash will embarrass the Science and Engineering Council (SERC), whose fixed budget is already under strain and which is already considering whether to strengthen support for general research at the expense, for example, of high-energy physics. Ironically, SERC finds itself facing the prospect of a shortfall in space funds at a time when an internal review group, under the chairmanship of Sir Mark Richmond, is about to recommend greater use of ESA by Britain's space scientists.

By the time science ministers meet in Paris to discuss the long-term goals of ESA, in January next year, Britain's European partners will expect a reaction to both new demands for funds. But, before then, the British Government needs to be convinced of the economic potential of a space station. At the DTI symposium, PA Technology attempted to do just this, presenting the results of a study on the indus-

trial processes that stand to benefit from a microgravity environment. It pinpointed two principal areas: the fabrication of defect-free semiconductor crystals and the preparation of extra large protein crystals. PA Technology says that the latter could benefit the enzyme business by allowing molecular structures to be defined quickly and efficiently.

Even so, British high-technology companies are sceptical of the benefits and of the speed with which they would emerge. The cost of the space station is estimated at \$8,000 million (1982 prices); the United States hopes that 15–20 per cent will be contributed by Europe and Japan.

PA Technology also argued at the DTI symposium that participation in the space station would stimulate industrial research in areas such as robotics, computer design and materials manufacture. British Aerospace has endorsed this view, presenting, at the DTI symposium, specific plans for an unmanned platform, launched by the US shuttle, to which Britain might contribute

40 per cent of the cost. This plan would complement rather than duplicate the US manned space station, says the company, which claims that the National Aeronautics and Space Administration has already responded enthusiastically to the suggestion.

Elsewhere in Europe, there are more subversive views. Strong bodies of opinion in West Germany and Italy are pushing for an independent European space station, perhaps to work in conjunction with a US platform. This idea, called Columbus, is being widely discussed within ESA and reflects a feeling abroad in Europe that greater autonomy is necessary. Buoyed by the successes of Ariane and Spacelab, many aerospace companies are eager to show that they can function independently of the United States.

The United States expects firm proposals for European collaboration in the space station by the end of 1985, and will want to know how the work would be divided between participating nations, which may include Japan and Canada as well as European states, acting either independently or in concert through ESA, by mid-1986.

Marcus Chown

UK Australian nuclear tests

Inquiry stirs fallen dust

ON 16 October, the president of the Royal Commission inquiring into British nuclear testing in Australia, Mr Justice James McClelland, urged that if the British Government wished to have its views accurately represented, it would be well advised to appear before the commission. The newly-installed British High Commissioner to Australia, Sir John Leahy, had earlier expressed concern about the recent public allegations that the British authorities had disregarded the safety of aborigines and atomic personnel.

The present official silence, Sir John said, must not be interpreted as tacit admission to the charges laid, that the decision to present evidence would be taken in London and that it would depend upon "how the commission goes about its business in future". Mr Justice McClelland replied that the Royal Commission could not be expected to forbid publication of allegations discomfiting to the British Government.

So far the allegations claim that unprotected servicemen flew sorties through radioactive clouds, that they decontaminated aircraft after the tests, that servicemen had buried the corpses of aborigines found on the range, that mentally-retarded civilians were placed in bunkers near a Maralinga blast in 1956 (see *Nature* 310, 90; 1984) and that servicemen were used as guinea pigs (see *Nature* 309, 199; 1984), a claim stressed by counsel for the British Nuclear Test Veterans Association.

On 3 October, the three royal

commissioners also heard an account of the discovery and attempted decontamination of a family of four aborigines who, it was alleged, had camped for a few days in the Marcoo bomb crater seven months after the 1956 Maralinga blast, in apparent contradiction to a report in January 1983 of the same event by the Australian Ionizing Radiation Advisory Council (AIRAC). Servicemen who had witnessed the event were told by a British officer that disclosure would result in court-martial and possibly the firing squad.

The commission's charter includes investigation of the safety measures taken before, during and after the tests, whether health had been affected, and the nature and size of the devices tested in the twelve major explosions and an unknown number of "minor trials" known as Rats, Kittens, Ayres, Tims and Vixens.

Last week, the federal government refused a request from the Australian Nuclear Veterans for legal aid to challenge the credentials of royal commissioner Mrs Jill Fitch. A conflict of interest arose, they asserted, from her continued membership of both the commission and AIRAC, since it was criticism by Professor Charles B. Kerr's committee in May this year of AIRAC's treatment of fall-out data which led to the commencement of the Royal Commission.

The commission is due to report in June 1985 and has applied to the British Government to take evidence in Britain later this year.

Jeffrey Sellar

European telecommunications

Community looks at strategy

Brussels

AFTER years of dithering, the member states of the European Economic Community have agreed to work together to strengthen Europe's telecommunications market. Meeting in Luxembourg on 15 October, industry ministers adopted two recommendations constituting a first step towards a telecommunications strategy.

First, all ten Community governments are to ensure that national telecommunications administrations consult before introducing new services. (The United Kingdom, in the throes of selling off half its nationalized industry, was at the outset rather embarrassed, but has now fallen in line.) Contacts already exist through the European Conference of Post and Telecommunications Administrations (CEPT) set up in 1959, but new services put into operation from 1985 would have to conform to standards now being laid down by the developing network of European standards bodies.

Six million ECU (European Currency Units) or \$4.6 million have been granted by the Community to these bodies for a two-year programme for 1984-85 to define and certify European norms in the telecommunications field. Equipment other than terminals, such as digital transmission and switching systems, would have to conform to common standards from 1986.

The second recommendation would throw open to all member states procurement of both conventional terminals (telephone apparatus for mains telephone use, PABX, ordinary teleprinters) as well as telematic terminals.

On switching and transmission equipment, and conventional terminal equipment for which there are no common specifications, the recommendation is that at least 10 per cent of the annual value of orders should be opened up for tender throughout the Community.

French pleas that the market should be opened to the full have been resisted by the European Commission, which is wary of risking a flood of components from both other member states and beyond, as well as scaring member states off a move it has taken them four years to make.

As a technical pioneer, and still the world's biggest exporter of first-generation telecommunications (telex, telephone), with a trade surplus of \$2,000 million a year, the Community recognizes its industry is at a turning point: how to develop a second- and third-generation market in telecommunications which can stand up to Japan and the United States.

The Community at present controls a 19 per cent slice of the world telecommunications equipment market, but growth to the end of the decade is forecast to be slower than in the rest of the world (6-7 per cent as opposed to 8 per cent in the United States and Japan). The Community's telecommunications sector represents 2 per cent of the Community's gross domestic product which, with innovation, could grow to 10 per cent by the year 2000.

The Commission, however, is looking further ahead. Telecommunications are already reckoned to affect 55 per cent of value-added services and 62 million jobs; according to the Commission, investments

in telecommunications could double the turnover of the European economy.

Under the Community's telecommunications programme, informally adopted by ministers last May, aid to finance telecommunications in ill-favoured regions could be drawn from the regional fund, the European investment bank and the new Community lending instrument (which is tailor-made to encourage investments in such conditions).

These schemes are likely to figure on the agenda of the next council of industry ministers on 20-21 November, when there will probably also be a decision on a programme of telecommunications research and development. Twenty-five million ECU a year over a five-year period has been proposed for Research in Advanced Communications in Europe (RACE), to be financed jointly by the Commission, companies and research institutes, much along the lines of the computer research programme Esprit.

Meanwhile, the Commission plans to set up a number of analysis and forecasting groups to look after the development of new services such as integrated digital networks, cellular radio-telephone services, videocommunications and wide-bank trans-national networks. At the October meeting, industry ministers also found an extra 11 million ECU to extend the 1979-83 programme on standardization in data processing for a further two years.

Anna Lubinska

North Sea records

A NEW centre for the storage of North Sea drilling records and cores was opened in Edinburgh last week by the junior energy minister, Mr Alec Buchanan-Smith. The centre will provide public access to core sections for sampling and inspection. Any oil company granted a drilling licence must make records and cores available, but only after an interval of five years.

According to Mr Buchanan-Smith, the first nine months of this year have seen more exploration and appraisal than any previous year. One hundred and forty wells had been drilled in the North Sea by the end of September, compared with last year's record total of 128.

The new centre will be administered on behalf of the Department of Energy by the British Geological Survey (BGS). The tasks of the 55 staff will include the provision of inspection facilities for the 40,000 core samples now housed, of which 50 per cent have been released for access. The associated borehole logs are stored in microfiche form. Samples of cores may be removed for experimental measurements subject to the approval of BGS, which says that the number of requests for access average about three a week. Apparently demand increases as deadlines for new licence applications approach. The annual cost of maintaining the centre is £120,000.

Philip Campbell

US policies offend Europeans

EUROPEAN optimism about the future of the new telecommunications strategy, as of other new industrial research and development programmes, is marred by anxiety about the effects of high-technology export restrictions by the United States.

There is speculation that the US 1976 Arms Export Control Act and the 1979 Export Administration Act will be tightened up by the new Congress when it re-assembles next January.

Existing controls, justified on national security grounds by the US Administration, are suspected of giving US companies an unfair advantage over others.

At the informal dinner following the Industry Council on 15 October, the soon-to-retire EEC Commissioner for Industry and Research, Vicomte Etienne Davignon, told national ministers that the European Commission intends to draw up an inventory of US measures to restrict high-technology transfer and to assess the consequences for economic and industrial development in the Community. The objective of the

study, which will be ready in six weeks, is to make it clear to the United States that security considerations and continued European economic and industrial development are not mutually exclusive.

There is particular concern in Europe at some of the recent measures by the US Administration to restrict the transfer of technology and the dissemination of scientific information, including restrictions on foreigners attending defence- or industry-oriented meetings and conferences, on the dissemination of certain published information, on the transfer of technical data regarding participants at private conferences to certain countries, stricter conditions for foreign scientists applying for entry visas and broadening the base for classified information.

More recently in Belgium, the United States attempted to block the export of agricultural machinery to the Soviet Union by the Pegard Company, on the grounds that it could be converted for military aims.

Anna Lubinska

Thermonuclear fusion

Europe from hand to mouth

THE 1982-86 budget of JET, the Joint European Torus for fusion research at Culham in Oxfordshire, is running out ahead of time. Planned to last 60 months, the budget will last for only 46, until October next year. The reasons are inflation (the 1982-86 programme was fixed at 1982 prices) and high maintenance costs at JET, including £250,000 to rebuild JET shock absorbers after an accident earlier this year.

Despite the economic crisis in the European Communities, which pay 80 per cent of JET's bills, there is little concern at Culham that the extra money will not be found. Community programme budgets never allow for inflation, says JET budget director Peter Kind, and it is always assumed that shortfalls will be made good in the next programme. Even if the 1985-89 programme (overlapping the first by two years), now before the council of ministers, is not agreed until next spring, JET accounts for 1985 could be balanced.

Mr Kind also has a contingency plan. Since operations began a year ago, JET has been short-staffed, so that there has been a squeeze on design time for the JET "extension to full performance" (neutral beam and radiofrequency heating, tritium handling equipment and so on). It has therefore been difficult to keep up the rate at which contracts have been put out to industry. Kind thinks it will be possible to let purchasing slip by a couple of months, particularly on items needed only some years hence.

The high maintenance and operating costs are exactly those predicted by the JET design team, but double what the council of ministers allowed in 1982. So JET can say "we told you so". "We had allowed 5 per cent of the capital equipment costs (excluding buildings) a year for maintenance", says Kind, but the ministers allowed only 2.5 per cent. But since JET began operations, there have been "10-15 requisitions a day for items from £50 to £5,000, from nuts and bolts to vacuum seals", reaching a total of around £10 million over a year, compared with JET equipment costs of £175-£200 million in today's values.

The largest item, the £250,000 for new supports, came after a single unfortunate discovery: that when the oval-cross-sectioned plasma ring was at its maximum elongation, the plasma snaked up and down and escaped control. In this shot, the plasma finally shorted out through the vacuum wall with currents of several megamps, and jolted the vacuum chamber with forces of hundreds of tons-weight. The force shook the floor like an earthquake, and although JET was not seriously damaged (it is now a millimetre longer than before) it has been thought prudent to modify its support structure.

Otherwise, JET has been running well, and made one particularly tantalizing discovery in the last two weeks of its recent closedown (to introduce neutral beam heating). The team has found that a carbide layer a few molecules thick can be deposited on the inconel inner surface of the vacuum chamber by making a glow discharge in a mixture of methane and hydrogen gas, and that this layer dramatically reduces the amounts of metal impurities entering the chamber from the wall. As these high atomic-number impurities efficiently radiate heat from the plasma, it is important to control their number.

Until this work, it had appeared that impurities would be a bigger problem at JET than expected. The carbide layer may bring a striking improvement. And because the layer can be efficiently removed, controlled experiments can be attempted.

Conservation in Japan

Modest gain for UK royal

Tokyo

PRINCE Philip, Duke of Edinburgh, has wrung some modest concessions on conservation from the Government of Japan. In his capacity as president of the World Wildlife Fund (see also *Nature* 18 October, p.595), the prince was last week promised that steps would be taken to restrict the import of endangered wildlife species into Japan. But the six-day visit did not yield all the prince had asked for.

In a 20-minute meeting with the prince, Japan's Prime Minister Yasuhiro Nakasone said that he would consider the introduction of domestic legislation to prevent the illegal import of protected species. A task force to study the problem is to be set up immediately. Customs officers are also to receive training in detecting illegal imports — the lists of endangered species are long and complicated and some dealers have skilfully forged documents.

The Minister for International Trade and Industry, Hikosaburo Okonogi, also promised to introduce by next April a system that will require dealers in endangered species to produce export permits from the governments of the nations from which the species are claimed to have come.

Rather less was promised though, in dealing with the vexed question of the 14 endangered species — six species of whale, three species of turtle, three species of monitor lizard, the musk deer and the African saltwater crocodile — which Japan refused to include in a list of endangered plants and animals and their products when it first became a signatory of the Convention of International Trade in Endangered Species, the Washington Con-

The effect was also seen earlier at the TEXTOR torus at Jülich, West Germany, but not followed up. In any case, it should not be over-emphasized, says JET physicist Alan Gibson.

Meanwhile, JET staff are crossing their fingers that Europe will come up with the cash JET needs. "JET is the only really successful example of European co-operation anywhere", claims Kind, "so there is a considerable political will to keep us going." Moreover, the present Irish presidency of the European Commission has put the 1985-89 nuclear fusion programme near the top of its priorities for agreement by the end of this year.

One explanation may be that Ireland sees JET as one of the few problems it can solve. JET officials, however, note uneasily that less senior Irish representatives in Brussels who have been discussing the fusion programme have been, with Greece, among the most negative. Who means what should be clearer at the November and December meetings of the research council.

Robert Walgate

efforts to reduce the number of species on the list, but gave no clue as to how or when this might be done.

Interest was also shown in the prince's desire to have Japan stage a conference on the protection of nature on islands. Among the prince's stops on his six-day tour of Japan was the island of Amami Ōshima, one of a group of remote islands known as the "Galapagos of Japan" and containing many endemic species of mammal, including the Iriomoto wildcat and the Amami rabbit.

Prince Philip had also expressed, in very diplomatic terms, his reservations about Japan's plans to continue sperm whaling in coastal waters, despite the International Whaling Commission's agreement to ban all sperm whale hunting. The Japanese whaling fleet, however, left harbour last Friday to hunt for sperm whales. The Japanese Government is now waiting to see what will happen once a whale is caught — will the US Government carry out its threat to cut Japan's fishing quota in US waters? Japanese officials had earlier expressed confidence that the United States would not retaliate even if whales were caught. Talks on the issue in Washington last week, however, were inconclusive.

Quite separately, the US Department of Commerce also announced last week that it would give Japan only a quarter of the fish quota in US waters it had requested for the rest of this year because of evidence that the Japanese fishing fleet had systematically violated earlier agreements. Whether such action will be extended if sperm whales are caught will depend on the strength of the US conservation lobby.

Alun Anderson

Nobel prize

Merrifield wins in chemistry

THOSE who have followed the growth of peptide research over the past twenty years will have received with delight and no surprise the news of Bruce Merrifield's Nobel Prize for Chemistry. The simple solid phase technique for the chemical synthesis of peptides which bears his name has been adopted in scores, possibly hundreds, of research laboratories worldwide. The concept has been widely applied in other areas of research, notably in oligonucleotide synthesis, with important consequences for genetic engineering, and in structural studies including protein sequencing.



To understand the impact that Merrifield's technique has made upon the development of peptide synthesis itself, it is necessary first to sketch in the situation in the 1950s and earlier. Peptide chemistry was then in an expansive mood. Natural peptides with powerful and important biological action, such as the pituitary hormone oxytocin, were being isolated. These natural peptides were soon shown to consist of short chains of the same building blocks (the α amino acids) as constitute the proteins. The sequence of amino acids in these linear chains was soon determined and the stage was set for the understanding of biological activity in terms of these relatively simple sequences. The techniques of peptide synthesis — the chemical union of α amino acids into chains of defined sequence — had been growing steadily during this period but were slow and labour-intensive. They could barely meet the explosive demand from pharmacologists and others for synthetic natural peptides and their structural variants which could be used to correlate chemical structure and biological activity.

It was just at this time that Merrifield introduced the simplifying step in peptide synthesis which rapidly transformed the situation. Much of the labour in contemporary peptide synthesis was involved in the isolation, purification and characterization of reaction products after each chain extension. Merrifield reasoned that by attaching the first amino acid to an insoluble polymeric support, these isolation steps could be much simplified and speeded up. There were other chemical

advantages which might also increase the efficiency of synthesis — a necessary concomitant since the polymeric system did not allow separation of the products of incomplete reactions. Most importantly, the process became amenable to mechanization.

Merrifield demonstrated these advantages convincingly in 1964 with a synthesis of the nonapeptide hormone bradykinin. The hormone was prepared singlehandedly in eight days, evidently in a highly pure form. By contemporary conventional methods, it could have occupied several skilled chemists for weeks or months.

The new technique of solid phase peptide synthesis was adopted with enthusiasm by biologists, sometimes beyond its immediate capabilities at this early stage in development. Organic chemists were slower to accept its advantages, some finding it in conflict with their rigorous classical training. Within the decade, the limitations and advantages were well recognized and solid phase peptide synthesis found and established a secure place alongside more conventional methods. The number of synthetic peptides prepared with its aid must now be counted in thousands.

By the mid-1970s, improved understanding of the solid phase system and numerous technical improvements allowed successful application to oligonucleotide synthesis. This was a timely development in view of the rapid strides in genetic engineering around this time. Again, synthetic DNA fragments became much more accessible using solid phase techniques. On the structure side, solid phase methods were developed for protein sequence analysis. Throughout organic chemistry, the concept of polymer-supported reagents and reactions has become commonplace. Although Merrifield confined his own activities to the peptide field in which he has continued to make massive contributions, many of these later developments must be attributed to his inspiration. The dream of automated synthesis of artificial proteins remains to be realized, but the impact of Merrifield's work is without question.

Robert C. Sheppard



Dr Carlo Rubbia, who shares this year's Nobel Prize for Physics (see p.701).

Animal welfare

Berkeley again in trouble

Washington

THE University of California at Berkeley is awaiting with more than the usual apprehension the result of an investigation by the US Department of Agriculture (USDA) into new allegations of animal welfare abuses. The reason: \$41 million of state funds earmarked for a new life sciences building cannot be spent until the university is able to satisfy the state legislature that there will be no future violations of the Animal Welfare Act. The building is already well behind schedule, and a decision by USDA to prosecute would further delay construction until the case had been decided.

Last July, the university was fined \$12,000 and ordered to correct long-standing deficiencies in its treatment of laboratory animals at the Berkeley campus (see *Nature* 2 August, p.356). The university was still under an order to "cease and desist" from future violations when the latest allegations were made on 10 September. Complaints were made to USDA by animal welfare groups, which entered animal houses "surreptitiously" and photographed a monkey with a tumour on its face and rabbits with local infections around cranial implants. The USDA investigation started after a surprise inspection on 12 September.

The complaint about the monkey has been dropped because USDA found no evidence of lapses in veterinary care. The care given to the rabbits is now being scrutinized, and a decision on whether to prosecute is expected before the end of the month.

Both the university and the researcher involved, Dr Walter Freeman, maintain that the animals were at all times under adequate veterinary care and that there were no violations. A university spokesman, Mr Debley, said the university "had been told" that the second complaint would also be dropped.

The requirement that the university must demonstrate compliance with the Animal Welfare Act before using state funds for the new building was written into California's 1984 Budget Act. A report by the university on the steps taken to improve animal care since the court case this summer is now being reviewed by the state legislature. Ironically, the building that may now be delayed is needed partly to improve the Berkeley campus's animal facilities. On 28 September, the university postponed an appearance before the state public works board where it was to have pressed its case for the new building. The university says the decision to postpone the appearance was due to "administrative delays" that had nothing to do with the latest allegations.

Tim Beardsley

Progress at NERC

SIR — As the new chairman of the Natural Environment Research Council (NERC), and after 8 years as a council member, I was intrigued to read your article (*Nature* 11 October, p.498) on the British Geological Survey and the related leading article (p.493) advocating the disbandment of the council.

While you have correctly identified some of the problems with which the council has grappled in recent years, I am disappointed that you have not recognized more fully the progress the council has made in the context of a severe shortage of funds.

The objective of a research council is to foster high-quality science in institutes and universities. Achieving this requires, firstly, well-motivated scientists and support staff, and secondly, money to pay them and supply the necessary support services and equipment. In spite of the council's strenuous efforts to persuade the government that environmental sciences need and deserve more funds, these have not been forthcoming on the science vote. Indeed, in the areas of NERC interest, with the exception of Antarctic research, science vote money has shrunk in recent years. Equally significant is the substantial reduction of income from commissioned research sponsored by government departments.

In the face of these reductions, my council has been at pains to maintain the level of its support for university research and has attempted to find new sources of commissioned research funds with the private sector. Contrary to your editorial comments, this has been done in line with principles clearly laid down by the Treasury, is largely on a collaborative rather than a competitive basis and is leading to the successful transfer of knowledge and technology from basic research to industry.

It will not surprise you or your readers that I reject your proposed solution to NERC's present difficulties and believe it would benefit neither science nor the country. It is a solution we have already examined and dismissed because we believe there is a real need within the United Kingdom for an organization which can promote objective environmental research to provide a basis for sound industrial, economic and political policies.

In taking up the office of chairman of NERC, I intend to continue actively the programme of reorganization and structural change which is already in hand and which, I believe, will provide purposeful support for environmental science research — basic, strategic and applied — of benefit to industry and government.

I trust that you and your readers will continue to show interest in the development of NERC and be prepared to recognize achievements resulting from reorganization as it proceeds. I believe that NERC has a strong and vibrant contribution to

offer the United Kingdom and the scientific community and that the environmental sciences deserves more than the emasculation you propose.

HUGH FISH

*Natural Environment Research Council,
Polaris House, North Star Avenue,
Swindon SN2 1EU, UK*

Telling all

SIR — In his article "Widespread after-effects of nuclear war" (*Nature* 23 August, p. 621), Dr Edward Teller points out that we should not expose the general public to highly speculative theories of worldwide destruction. Instead we should tell people exactly what we know and do not know about the nuclear winter, "for only then can decisions affecting the well-being of Western societies be made on an intelligent basis". I think that the general public would be even more satisfied if the decisions could consider the well-being of all societies.

TOM REUTER

*Department of Zoology,
University of Helsinki,
00100 Helsinki, Finland*

US deficit

SIR — Your leading article "Election irrelevant" (13 September, p.92) wonders about the arithmetic of funding the US federal budgetary deficit.

In the fiscal year ending 30 September, this will be \$172,000 million¹, but the trade deficit this year may exceed \$130,000 million². The dollars expatriated on current account are therefore sufficient to cover 130/172 or 75 per cent of the budgetary shortfall if reinvested on capital account directly or indirectly in American government securities.

BRIAN POTTER

*3305 Pottawattamie Trail,
Michigan City, Indiana 46360, USA*

1. *The Economist*, 15 September, 1984, p.24.
2. *Ibid.*, p.18.

Miracles

SIR — Berry *et al.* (see *Nature*, 19 July, p.171) must be born-again Baconians. This conclusion can be drawn from their statement that the laws of science are generalizations of experience and therefore cannot be applied to the examination of claims for miracles. Falsificationists would define the accumulations of scientific laws in terms of hypotheses that have so far survived empirical testing.

This subtle difference in emphasis suggests that the debunking of miracles is a job for a falsificationist whereas a neo-Baconian may be permitted the luxury of faith without being faced by a dilemma. It is however interesting to note that in fact

professional illusionists appear to be better investigators of miracles than scientists, if the Yuri Geller investigation can be regarded as an exemplar of a rational examination of a claim for supernatural powers.

Berry *et al.* may therefore have arrived at a correct conclusion but for the wrong reasons. Alternatively, professional magicians perhaps may be regarded as conducting scientific research in the Popperian tradition when employed in the investigation of miracles.

BARRIE PEARSON

13 Knoll Cottage

*Residential Park,
Winfrith, Dorchester,
Dorset DT2 8LD, UK.*

Enough of Homer

SIR — M. Pulbrook (*Nature* 309, 204; 1984), focusing on a parenthetic point I made within a quote-marked hypothetical question (*Nature* 307, 590; 1984), has produced an interesting exegesis on black versus red Homeric wine — but the contrast I had in mind was red/white, or yellow. Compare the classicist W.B. Stanford, *Commentary on Odyssey*, 1947, Vol. 1, p. 355, line 196, Bk. IX: Homer "never mentions white or yellow wines". And *pace* Pulbrook, I did not assume that the key question in the wine-dark sea controversy is to "account for the redness of the sea": this surely is evident from my mention of many other aspects of the topic, formulaic devices, for example.

As the distant Homeric world-view and pattern of sensibility differ significantly from ours, our thinking about Homer's epics is easily subject to various colourations. And whatever the pros and cons of translating οἶνον πικρὸν as "wine-dark sea", the English phrase itself is viable and poetically very potent: to exemplify a facet, Kazantzakis once envied the relative monosyllabicity (!) of English.

This interdisciplinary international correspondence has been piquant. *Floreat Nature* . . .

MICHAEL MACNAMARA

*Philosophy Department,
University of South Africa,
PO Box 392,
Pretoria 0001, South Africa*

SIR — Regarding your editorial note about this correspondence (*Nature* 12 July, p.95), the *literati* used to believe that the last word came from Stephen Dedalus when he spoke the winged words "snotgreen and scrotum-tightening sea" (Joyce, *J. Ulysses*, Bodley Head, London, 1937).

Thank you nevertheless for your nostalgic revival of a dying culture, so aptly rounded by a scholarly note from Balliol.

HILTON STOWELL

*ERBP Laboratory,
120 Nature Creek SW,
Milledgeville, Georgia 31061, USA*

● This correspondence is now closed — Editor, *Nature*.

CERN's first Nobel prize

Stockholm's recognition of the importance of the discovery of the intermediate vector bosons W and Z is nicely tempered by an appreciation of the technical ingenuity which underlies it.

CARLO Rubbia, Italian-born discoverer of the W and Z particles that carry the weak nuclear force, and Simon van der Meer, the Dutch accelerator designer without whom, says Rubbia, "we couldn't have done it", share the 1984 Nobel Prize for Physics. But neither is sitting back. They are, instead, separately at work on the proton-antiproton collider at the European Organization for Nuclear Research (CERN) near Geneva that won them the prize.

Rubbia is at present working flat out to exploit the collider to its utmost. There are two objectives. While the approximate masses and decay properties of the W and Z particles are now known, there is much detail yet to discover — such as the particles' lifetimes. Clearly, CERN would be well-advised to wring as much as possible from the machine before Stanford's Single Pass Collider (planned to produce a million Zs a year, compared with CERN's present few dozen) comes on line towards the end of 1987. But Rubbia and his colleagues have also been stimulated by hints from his "UA1" detector on the collider that all is not quite as expected at energies beyond the W and Z particles.

"Our chance is now" he says. Rubbia is pleased at finding the Ws and Zs, but clearly feels like an explorer who arrives at the South Pole and finds footprints in the snow — here, the footprints of Abdus Salam, Steven Weinberg and Sheldon Glashow whose "electroweak" unification predicted the presence and exact properties of the particles. "For me these anomalies — and I must be frank with you, we're not sure they're there — represent hope", he said last week. "We may be on the threshold of a whole new territory."

Rubbia has been the driving force behind the collider. "The road was very long", he said. "You have to be a hell of a persistent person. You have to be very stubborn." With two American colleagues, he first presented the idea, in 1976, to Robert Wilson, then director of Fermilab near Chicago, who rejected it. Rubbia, ebullient and outspoken, feels the reasons were partly personal. "I didn't get on with Wilson. He'd had enough of me." Fermi-

lab went ahead instead with its Tevatron project, an upgrade to 1,000 GeV beams. (This was a lengthy project, involving the development of superconducting magnets, and it will not provide competition for the CERN collider before 1986–87 — when it will collide protons and antiprotons at 2,000 GeV total energy.)

The Rubbia project went ahead instead at CERN, assisted by the better vacuum in the CERN super proton synchrotron, through which the colliding proton and antiproton beams now circulate. In the poorer Fermilab vacuum, the beams would have been rapidly damped, and a new beam-tube would have been necessary. CERN also had van der Meer ("our best accelerator man"), whose essential contribution to the collider has been "stochastic cooling", a technique which allows an accumulation of antiprotons to collide with protons in the collider.

Stochastic cooling is much more than a technical *tour de force* but, on the face of things, is a violation of Liouville's theorem of statistical mechanics describing the motion of collections of particles. Only once before has a Nobel prize been awarded to an accelerator physicist — Ernest O. Lawrence, for his design of the cyclotron. The comparison of van der Meer with Lawrence is a measure of the stature of the Dutchman's achievement.

The problem van der Meer has solved is this. Antiprotons are formed in high-energy collisions of a proton beam with a target, and collected into a rough beam. But the particles so produced have a wide spread of velocities, and so could not be accelerated coherently in a synchrotron machine. The velocity spread has to be reduced or, in the jargon, the beam has to be "cooled". An earlier (1960s) Soviet solution (due to the late Gersh Budker of Novosibirsk) was to inject a "cool" (mono-energetic) beam of electrons to run alongside the protons: the electrons would "heat up" and the protons "cool" until the beams reached the same "temperature" (this thermodynamic analogy is exact). Then further cooling would be done with a new injection of electrons until the proton beam was sufficiently cool that it could be accelerated up to the 270 GeV planned in the collider (the super proton synchrotron). This idea worked, but proved too complicated to use in practice.

van der Meer's approach is different. Liouville's theorem has it that the crowd of points representing a collection of particles

in phase space (embodying both momentum and position) will keep a constant density, whatever the external forces acting on it. According to Liouville, a beam is like toothpaste in a tube: if you squeeze it somewhere in phase-space, it must come out somewhere else. But "cooling" aims to increase phase-space density, by putting all the particles in a physic bunch in real space, each with the same momentum. How does van der Meer's cooling method get around this difficulty?

"I began thinking that if you can control a single particle perfectly in an accelerator, then why can't you control several?" he says. He developed the idea that an uncooled beam of particles is represented in phase space by points (one for each particle) with plenty of space between them, and that in principle these points can be squeezed closer to each other without changing the phase-space density around each point.

In practice, the technique is to control the fluctuations of the beam. Antiprotons are accumulated in a storage ring called the Antiproton Accumulator. Sensors detect deviations at one point on the ring, ultra-fast electronics calculate the necessary corrections and correcting signals are relayed to some point later in the trajectory. The process is possible — with both beam and signal travelling near the speed of light — because a chord of a circle is shorter than the circumference which it intercepts. This whole correction process takes a few tens of nanoseconds, and is repeated for each circuit of the beam. Roughly 24 hours are needed to accumulate and cool enough antiprotons for a collider run, but van der Meer is working on a new accumulator (ACOL) that will reduce this by a factor of ten.

ACOL will take its first beam only in May 1987. Meanwhile, Rubbia and his 130-strong UA1 collaboration* will try to get as much time and energy as possible to explore the "new territory".

Robert Walgate

Erratum

IN the penultimate paragraph of Stephen Budiansky's article "Jumping the smoking gun" (*Nature* 4 October, p.407), the statement that "none of the bacteria residing in the gut of farm animals is resistant" should have read "not all of the bacteria...".

*The UA1 group includes teams from West Germany (Aachen and Kiel), Austria (Vienna), CERN, the United States (Harvard, Riverside and Wisconsin), Finland (Helsinki), Italy (Padua and Rome), France (Annecy, Collège de France and Saclay), the Netherlands (Amsterdam) and the United Kingdom (Birmingham, Queen Mary College London and the Rutherford-Appleton Laboratory).

Photoreception

Bovine-like rhodopsin in algae

from Howard C. Berg

THE outer segment of the light-sensitive retinal rod has long been the system of choice for work on phototransduction in vision, from the isomerization of 11-*cis* retinal to the transient closure of sodium channels in the plasma membrane. Recent advances have centred on amplification involving GTP-GDP exchange, on activation of phosphodiesterase and on hydrolysis of cyclic GMP (Stryer, L. *Cold Spring Harb. Symp. quant. Biol.* **48**, 841; 1983). To those of us who work on sensory transduction in microorganisms, for example on chemotaxis in bacteria, it seems remarkable that so much has been learned without the use of genetics. There is nothing like the right mutant to open the door to fresh discovery, to reveal an unsuspected component or process essential to the function of a complex biological machine. The report on page 756 of this issue that the green alga *Chlamydomonas* can see with the aid of a bovine-like rhodopsin foreshadows a renaissance in studies of eukaryotic photoreception.

Chlamydomonas is an aquatic organism about 10 μm in diameter that is propelled along a helical path by means of two anterior flagella. For photosynthesis, the cell uses blue and red light, which it detects using one chloroplast while for phototaxis, it uses blue-green light, which it detects with one lateral eye, or eyespot. It swims towards or away from a source of light, aligning the axis of its helical path with that of the beam. In 1980, Foster and Smyth argued that the eyespot is a directional antenna, comprising alternate layers of material of different refractive index, that enhances contrast on a layer of photoreceptor pigment in the overlying plasma membrane (*Microbiol. Rev.* **44**, 572). They noted that this antenna completes a 360° lateral scan of the environment every time the cell advances one helical turn. Using a phase-sensitive system that causes one flagellum to pull harder than the other when the output of this scan is non-uniform, the cell can correct its course and 'lock on to' the beam. On the basis of the phototactic action spectrum and other optical properties of the cell, they predicted that the photopigment would be a bovine-like rhodopsin.

Now Foster and his associates, using retinal analogues provided by Nakanishi's group, have proved the point. Phototaxis of a carotenoid-free mutant is restored on addition of suitable analogues, and the shifts in the action spectra parallel those seen in absorption spectra of bovine rhodopsins. The pigment protein clearly differs from bacteriorhodopsin, which works with different analogues and has

different spectral properties.

Most steps of the sensory pathway remain to be worked out. Coupling to the flagella presumably occurs via changes in membrane potential, since such changes are observed, with the appropriate action spectrum, when a related green alga, *Haematococcus*, is exposed to light (Litvin, F.F., Sineshchekov, O.A. & Sineshchekov, V.A. *Nature* **271**, 476; 1978). The two flagella of *Chlamydomonas* have different sensitivities to calcium ions (Kamiya, R. & Witman, G. B. *J. Cell Biol.*

98, 97; 1984), so voltage-sensitive calcium channels might be involved. Little is known about how light absorption generates changes in membrane potential. It is at the level of these early steps in phototransduction that we can expect to see the most significant advances.

Lest anyone should doubt the power of genetic and biochemical dissection in *Chlamydomonas*, look how it has broadened our understanding of the assembly, structure and function of the eukaryotic flagellum (Luck, D.J.L. *J. Cell Biol.* **98**, 789; 1984). Similar breakthroughs should now be possible in studies of eukaryotic photoreception. □

Howard C. Berg is in the Division of Biology, California Institute of Technology, Pasadena, California 91125.

Palynology

Andean guide to Pliocene-Quaternary climate

from J.R. Flenley

THE city of Bogota, 2,600 m up in the Colombian Andes, enjoys a remarkably level site, being built on the dry bed of a former lake. A resistivity survey has recently shown that the lake deposits are 800 m deep and have probably been accumulating continuously from well back in the Pliocene and throughout the Quaternary. This has provided a unique opportunity to obtain a complete land-based record of the climatic oscillations of the Quaternary to complement the existing deep-sea record. The results are contained in *Vegetational and Climatic History of the High Plain of Bogota, Colombia: a Continuous Record of the Last 3.5 Million Years* (Cramer, Liechtenstein; 1984), by H. Hooghiemstra.

The deep-sea record has been investigated by Nick Shackleton from Cambridge, and Neil Opdyke at Lamont-Doherty Geological Observatory. By oxygen isotope analysis of the shells of foraminifera from the ocean-bed ooze they have shown that there were numerous major climatic cycles in the past 1.6 Myr, that is since the Olduvai magnetic event which provides one possible definition of the Pliocene-Quaternary boundary. Oxygen isotopes, however, though giving an indication of the amount of ice in the world and of the palaeotemperature of the ocean surface, tell one little about ecological conditions on the continents. In that respect the continuous land-based record from Bogota is very important indeed.

The Bogota sediments have now been cored to a depth of 357 m. Fortunately they contain numerous bands of volcanic ash, which can be dated by the potassium-argon method, allowing it to be established

that this part of the record covers the last 3.5 Myr. The monumental task of pollen-analysing this core fell to Henry Hooghiemstra and O.K. Hulshof, working with Tom van der Hammen at the Hugo de Vries Laboratorium in Amsterdam. After 1,230 pollen samples and 378 pollen and spore types, they graphed their results with a laser-beam plotter.

The resulting diagram has been divided into no less than 55 zones, representing 27 complete major climatic cycles, which can be correlated with those from the deep-sea record, and extend it much further back in time. The fluctuations in mean annual temperature are from <6°C to >15°C—so much for those who would write off Quaternary temperature change in the tropics. Some of the most marked fluctuations and some of the coolest temperatures occur in the Pliocene part of the record. It seems that the Late Tertiary was not the time of gently declining temperatures which it was once thought to be.

The great advantage of a land-based core over an ocean core is that it gives direct biogeographical and ecological information. The Isthmus of Panama and the Andes were formed relatively late in the Tertiary, before which it was almost impossible for taxa from North America to migrate into South America. In the Bogota record, we can actually see the arrival of the North American trees *Quercus* and *Alnus*. *Alnus* arrived in the Late Pliocene, about 2.9 Myr ago; *Quercus* appeared on the scene only 1.0 Myr ago. Such direct evidence of migration provides a factual basis for biogeography and offers essential tests for the hypotheses put forward by students of modern biogeographical distributions.

The major ecological cycle recorded is the alternation of rain forest elements with those from the paramo, the grass-dominated vegetation above the tree line. It is these alternations which provide the evidence for temperature fluctuation. Careful inspection of the pollen curves shows that no two cycles are precisely the same. *Alnus*, after its arrival, usually dominates the forest pollen, but the proportions of other forest trees such as *Podocarpus*, *Weinmannia*, *Hedyosmum*, *Rapanea* and *Miconia* vary rather widely and are constantly fluctuating. Some of these variations may indicate hydrological differences between the forest periods, but others could relate to the population biology of individual taxa or to complex ecological interactions. Two points emerge: there is no set pattern of succession in each forest period and there is no stable 'climax'. In fact the diagram is more an exhibition of continuous change than of any kind of stability; any idea of the rain forest as a stable museum-piece does not apply to these montane forests.

Is the Bogota record unique or can we hope to find similarly long undisturbed records elsewhere? In glaciated parts of the world it is clearly unlikely and in subtropical arid regions deposition may often have been interrupted by aridity and by deflation of sediments. It is therefore no accident that this valuable record is from the tropics. The Bogota lake happens to be dry, but there are other tropical lakes which are wet and almost certainly contain long records. The most likely examples are the East African Rift Valley lakes, although the tectonic lakes of the Sumatran Rift Valley also hold out much promise. What is needed now is an international coring project for tropical deep lakes. A start has been made by Dan Livingstone of Duke University, who plans to core the meteorite crater of Lake Bosumtwi in Ghana, and it would be tragic if the expertise to be gained so expensively in money and time at Bosumtwi were not to be more widely used.

The amount of laboratory work involved in analysing the Bogota core was enormous but that being generated by analysis of a 1,000 m core from Lake Biwa in Japan is positively daunting. How incongruous that the pollen analysis of cores obtained with such sophisticated technology is carried out with 'steam-age' manual preparation and optical microscopy. This is true not only for pure research, but also in the field of oil search where palynology is increasingly important. It seems odd that so few palynology laboratories put any effort into modernizing their techniques, and that research-funding bodies have not yet supported attempts to apply information technology in this challenging and commercially significant area. □

J.R. Flenley is in the Department of Geography, University of Hull, Hull HU6 7RX.

Evolution

Creationism in confusion

from Michael E. Howgate and Alan J. Lewis

THIS Saturday (27 October, 2.30pm, Conway Hall, Red Lion Square, London WC1) there is to be a debate between the Association for the Protection of Evolution (APE) and the Biblical Creation Society (BCS) whose talents were on display at an open day during the society's biennial conference*.

Evidently, BCS members range from 'young earthers' who are strict literalists and believe that the Earth was created in six days about 6,000 years ago, to 'mature earthers', who believe it was created about 6,000 years ago but looking much older. It even includes some who put forward the 'gap' theory, which proposes that geology happened sometime between the Fall of Adam and the Flood.

The open day was introduced by David Tyler, who set the tone by being very non-committal about whether Hutton's classic unconformity at Siccar Point was due to the Flood. However, he was convinced that the presence of a basal breccia and a smoothly planed-off contact was due to some unnamed catastrophic event. Tyler is unusual for a creationist in that he does go into the field to look at the evidence, although he freely admits that his observations are interpreted in the light of 'the biblical constraints on (his) geological thinking'. Tyler, a young-earth theorist (and an organizer of the Open University Geology Club), disparages Uniformitarian geologists for relying on present-day observable analogies when interpreting past processes, but seems not to be averse to doing the same when using the Mount St Helens eruption as a convenient catastrophist analogy. As he is faced with a multiplicity of non-correlatable unconformities throughout the geological column, Tyler seems destined to arrive at the position of Baron Cuvier who, nearly two centuries ago, saw the necessity to interpose as many catastrophes as required in order to match up his catastrophism with the record of the rocks.

A surprising fillip for evolutionists was the discovery that BCS's top geneticist, Chris Darnbrough (Glasgow University) believes in evolution. However, his lecture *Genes, created but evolving* gave the game away. Far from his belief in the genetic mechanisms of evolution affecting his literalist faith in creation, Darnbrough believes that the 'Biblical kinds' of organisms have evolved, but only since the Flood.

In his keynote theological policy statement, Edgar Andrews, professor of materials at Queen Mary College, London, wisely distanced the BCS from US

creationism, which he described as being ascriptural and anti-scriptural, as well as suffering from inconsistency. Andrews condemned the opportunist tactics of 'scientific creationism' for leaving the revealed word of God — the Bible — out of their arguments. More specifically, Andrews did not criticize the pseudo-science of the American creationists but concentrated his attack on their failure to appreciate fully the role of Christ Himself as the agency of Creation.

David Gower, reader in biochemistry at University College, London, gave a biblically uninterpreted account of recent advances in the study of olfaction. But in the finale to his lecture he opined that nasal biochemistry was firm evidence for creative design as opposed to natural selection. When tackled on the subject of inherent and genetic defects in olfaction and gustation as well as the lustful temptations presented by pheromones, Gower unenthusiastically admitted that his theological position impelled him to ascribe these faults to the Fall of Adam or to the agency of the Devil.

When a stalwart of the rival creationist organization, the Creation Science Movement, attacked the backsliding into theism of the BCS and asked for a show of hands in support of young-earth, six day, literalism, only one member of the panel of lecturers committed herself to this position. (Perhaps the presence of your Neodarwinist correspondents had an inhibiting effect on their forthrightness). It is to be hoped that at Saturday's debate with APE, the BCS protagonists will not hide the flame of their empirical predictions behind a bushel of theological diversions. □

Michael E. Howgate of the Department of Zoology, University College London, WC1E 6BT and Alan J. Lewis of the Department of Electrical and Electronic Engineering, Brunel University, Uxbridge UB8 3PH, are founder members of APE.

100 years ago

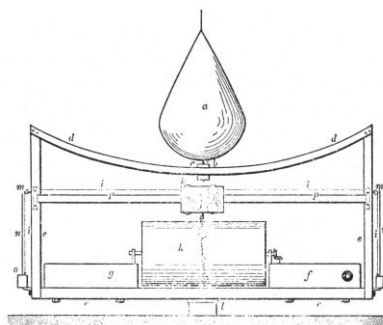


Fig. 4 Horizontal component of wave-path register for strong and destructive earthquakes.

From *Nature* 30, 610, 23 October 1884.

*'Creation and Origins Study Conference', High Leigh, Hoddesdon, Herts, 23-26 July 1984.

Anthropology

Supertramps at sea

from Jared M. Diamond and William F. Keegan

WHEN European navigators began 'discovering' the rest of the globe in the fifteenth century, they were surprised to find that technologically less advanced peoples had already settled many oceanic islands. The pattern of settlement is intriguing: in the tropical Pacific almost every habitable scrap of land, no matter how small or remote, was occupied, whereas in other oceans much larger and less remote islands, for example the Madeiras, Azores, Mauritius and Flinders, remained vacant. Recent archaeological work has shown that Pacific peoples colonized earlier, farther and at a lower level of overall technology than did Europeans, Africans and Amerindians. It is now possible to explain why this pattern should be so, and to draw interesting parallels between island colonization by man and that by birds and other animals.

While most Atlantic islands remained empty until thousands of years after the rise of Western civilization, stone-age hunter-gatherers reached the eastern Indonesian islands of Flores and Timor by 100,000 yr BP (ref. 1), Australia and New Guinea by 50,000 yr BP (ref. 1), the Bismarck Archipelago by 11,000 BP (ref. 2) and the West Indies around 5,000 yr BP (refs. 3, 4). Madagascar was colonized by people from remote Indonesia rather than from nearby Africa, while the colonists of Hawaii and Easter Island stemmed ultimately from the west rather than from the closer Americas. The explanation for this unexpected pattern of settlement, put simply, is that the Pacific is endowed with far more islands than are other oceans.

One reason why both the area and distribution of islands are important factors for colonization is that close islands serve as stepping-stones to more remote islands. For instance, Polynesians or Caribbean Indians did not reach Easter Island or Cuba in a single leap from New Guinea or Venezuela, but via many intermediate islands. But stepping stones cannot be the whole explanation. To reach remote Pacific islands such as Easter Island, New Zealand and Hawaii, Polynesians had to cross stretches of open ocean of up to 3,500 km (refs 5, 6), far greater than the distances from the nearest mainland to the Atlantic and Indian Ocean islands that Africans and Europeans failed to reach until modern times. This suggests that the availability and initial discovery of many habitable Pacific islands stimulated overwater colonization by humans. For similar reasons the bird species of New Guinea include many more superior overwater colonists than do the birds of South America or Africa. Thus, understanding of island colonization by humans^{7, 8} raises

problems similar to the island biogeographical problems that have long fascinated students of plant and animal distributions⁹.

For European navigators the effect of island availability on colonizing effort is obvious. Between 1400 and 1800 the discovery and expectation of habitable land catalysed further voyages dedicated to exploration and settlement. Egocentric Europeans have tended to assume, however, that only they explored consciously, while illiterate stone-age peoples must have reached islands by accidental drift voyages¹⁰. Archaeologists still believe this theory holds for the settlement of Australia



Portrait of a New Zealand man by F. Parkinson, on Cook's first voyage, 1768-71 (British Library). and New Guinea from Indonesia. The first securely dated human remains from Australia/New Guinea (united to each other in the Pleistocene) date from around 50,000 yr BP (ref. 1), and there is no proof of a second wave of human colonization until the arrival of pigs, taro and dogs of Asian origin around 5,000-10,000 yr BP. In this case, the simplest interpretation is that one raft-load from Timor or the Moluccas survived after being swept to Australia/New Guinea¹¹, and that a similar accident did not happen again for 40,000 yr.

By contrast, for the Mediterranean and

Polynesia, archaeological research has shown that colonization could only have been purposeful. The presence of obsidian from the Aegean island of Melos on the Greek mainland demonstrates overwater voyaging in the eastern Mediterranean around 13,000 yr BP (ref. 12). Yet mainlanders did not choose to begin settling eastern Mediterranean islands until the rise of agriculture and domestication first made self-sustaining human populations possible on the largest islands around 8,000 yr BP. Settlement gradually spread to smaller Mediterranean islands as subsistence techniques improved over the following five millennia¹².

In Polynesia, not only settlement but also discovery of many islands must have resulted from purposeful voyaging. Computer simulations and historical records show that there is essentially no chance of eastern Polynesia having been reached by accidental drift voyages^{13, 14}. Detailed studies of Polynesian navigational techniques¹⁵, and the Hawaii-to-Tahiti sea trial of the reconstructed Polynesian canoe Hokule'a¹⁶, have demonstrated that the exploration of the Pacific was within Polynesian voyaging capabilities. Polynesian islands were discovered during a series of upwind voyages that reached Fiji, Samoa and Tonga slightly before 1000 BC, the Marquesas around AD 300, and all other Polynesian islands over the following 500 yr (refs 5, 6). Human introductions of crops and domestic animals throughout Polynesia provide abundant evidence that settlement as well as discovery was planned¹⁷.

All this is not to say that prehistoric navigational skills were such as to ensure risk-free exploration and colonization. On the contrary, the settlement of Polynesia is estimated to have cost 500,000 Polynesian lives at sea, a number equal to Polynesia's standing population once settled⁵. The motivation of prehistoric voyagers was strong, however. Those few fifteenth and sixteenth century European navigators who returned alive told tales of wealth and habitable lands that triggered an explosive exodus of Europeans seeking these and other still-undiscovered lands. Similar reports of discovering empty islands must have driven the explosive wave of colonization in Polynesia between AD 300 and 800. With the occupation of all the Pacific's habitable islands, the main motivation for long-distance voyaging ceased, and it was only a memory by the time Europeans reached Polynesia.

- White, J. & O'Connell, J. *A Prehistory of Australia, New Guinea, and Sahul* (Academia, Sydney, 1982).
- Specht, J., Lilley, I. & Normu, J. *Aust. Archaeol.* 16, 92 (1983).
- Rouse, I. & Allaire, L. in *Chronologies in New World Archaeology* (eds Taylor, R. & Meighan, C.W.) 431 (Academic, New York, 1978).
- Maggiolo, M. & Vega, B. *J. New Wild Archaeol.* 5, 33 (1982).
- Jennings, J. *The Prehistory of Polynesia* (Harvard University Press, Cambridge 1979).
- Kirch, P. *Adv. Wild Archaeol.* 1, 51 (1982).
- Jones, R. in *Sunda and Sahul* (eds Allen, J. et al.) 317 (Academic, London, 1977).
- Terrell, J. *Wild Archaeol.* 9, 62 (1977).
- MacArthur, R. & Wilson, E. *The Theory of Island Biogeography* (Princeton University Press, 1967).

- Sharp, A. *Ancient Voyages in Polynesia* (Angus & Robertson, Sydney, 1963).
- Birdsell, J. in *Sunda and Sahul* (eds Allen, J. et al.) 113 (Academic, London, 1977).
- Cherry, J. *Proc. Prehist. Soc.* 47, 41 (1981).
- Levinson, M., Ward, R. & Webb, J. *The Settlement of Polynesia: A Computer Simulation* (University of Minnesota, Minneapolis, 1973).
- Dening, G. in *Polynesian Navigation* (ed. Gordon, J.) 102 (Polynesian Society, Wellington, 1963).
- Lewis, D. *We, The Navigators* (University Press of Hawaii, Honolulu, 1972).
- Finney, B. *Science* 196, 1277 (1977).
- Kirch, P. *Archaeol. Oceania* 17, 1 (1982).
- Diamond, J. & Marshall, A. *Emu* 76, 187 (1976).
- McGhee, R. *Am. Antiq.* 49, 4 (1984).

The patterns of distribution of Polynesians in the eastern and western Pacific contrast dramatically⁵. On archipelagos lacking other peoples (from Tonga eastwards, including New Zealand), the Polynesians occupied all islands. On tropical western Pacific archipelagos from the New Hebrides to the Carolines, which are shared with Melanesians and Micronesians, the Polynesians occupied only small or outlying islands. In the Solomons, for instance, Polynesians were confined to Rennell and six other outliers, while Melanesians were on all other islands. How could Polynesians have swept thousands of miles across the Pacific from eastern Polynesia to New Zealand and Rennell, without completing the last jumps of 1,000 and 100 miles respectively to Australia and the central Solomons?

The answer proves to be that Polynesians were excluded by established

human populations on Australia and western islands other than small outliers: the Rennellese who reached the central Solomons are known to have been killed and eaten. For Australia, a similar exclusion is inferred from Polynesian-style stone axes found along the east coast. The distribution of Polynesians on Pacific islands is paralleled in detail by distribution of numerous bird, bat, lizard, snail and plant species (termed supertramps), which are similarly confined to outlying islands in western archipelagos by established related species¹⁸. Other cases of human supertramps excluded from mainlands are the Norse from Newfoundland by Amerindians¹⁹ and West Indian Arawaks from Florida by other Amerindians. □

Jared M. Diamond is in the Department of Physiology and William F. Keegan in the Department of Anthropology at the University of California, Los Angeles, California 90024.

Astronomy

Chaotic spinning of Hyperion?

From Carl D. Murray

FOR many people the Solar System is a paradigm of ordered regular motion. Indeed, the origins of mechanics can be traced to the early attempts to explain the observed regularity in the motions of planets and satellites. However, according to recently published work by Jack Wisdom and Stanton Peale of the University of California, Santa Barbara, and François Mignard of CERGA, Grasse, the Solar System probably contains one permanent member, Saturn's satellite Hyperion, whose rotational behaviour is far from regular (*Icarus* 58, 137; 1984).

A general feature of chaotic motion is that relatively simple equations can give rise to solutions with complicated unpredictable behaviour, the degree of chaos depending on a number of factors. The common analogy of a deterministic yet chaotic system is a pinball machine, where small changes in starting conditions can produce radically different results. Hyperion's orbital motion and its interaction with the satellite Titan are quite well understood, and apparently regular. What Wisdom, Peale and Mignard have investigated is the effect of Hyperion's unusual shape on its spin behaviour.

Most of the natural satellites in the Solar System exhibit what is known as synchronous spin-orbit resonance, that is, the satellite's spin period is approximately equal to its orbital period. The most familiar example of this configuration is the Earth-Moon system, resulting in the Moon always presenting the same face to the Earth. Such configurations are not due to chance but have arisen because of the effects of tides raised on the planet by the satellite, the time scale for the process depending on the mass of the satellite and

its distance from the planet. For Hyperion, which is small, this time scale was known to be a significant fraction of the age of the Solar System, even before the Voyager missions, but observations had not produced a definitive spin period. In 1980 and 1981 Voyager images of Hyperion revealed a satellite with a highly irregular shape which has been variously described as resembling a cigar or hamburger (see the figure). Wisdom *et al.* show that it is this unusual shape which will probably prevent Hyperion from ever achieving a stable spin-orbit configuration.



Three images of the saturnian satellite Hyperion from Voyager 2 spacecraft. (JPL and NASA.)

A spin-orbit resonance can occur when the ratio of the spin period to the orbital period is some simple fraction. Near-spherical objects can be trapped in a number of different spin-orbit resonances other than the synchronous one — the planet Mercury is actually in a 3/2 spin-orbit resonance. Each resonance occupies a well-defined region in the phase space and has a narrow chaotic region at its boundary. Only in this narrow region does the behaviour of the spin become unpredictable. If the satellite shape is irregular, with large differences in the principal moments of inertia, then nearby resonances start to overlap and the extent of the chaotic region dramatically increases. A criterion for the onset of chaos as a result of overlapping resonances was

first developed by Chirikov (*Phys. Rep.* 52, 263; 1979) in connection with work on plasma confinement. With parameters appropriate for Hyperion, Wisdom *et al.* show that the chaotic region around the synchronous state is so large that the 3/2 state vanishes and the 1/2 and 2 states are reduced to small islands of apparent stability in a sea of chaos. If Hyperion finds itself in this large chaotic region, its spin period will undergo essentially random variations over a few orbital periods. Nevertheless, Hyperion could still survive in the synchronous state provided that its spin axis remained perpendicular to the orbital plane. But Wisdom *et al.* have also carried out an attitude stability analysis and shown that the synchronous and 1/2 states are unstable to small displacements, resulting in a chaotic tumbling motion. Hyperion's only hope for a chaos-free existence is to enter the attitude stable 2 state. The probability of such an event is very low, however, so Wisdom *et al.* conclude that Hyperion will be found to tumble chaotically.

This naturally leads to the question of what evidence is available in the Voyager images to support a claim for the chaotic tumbling of Hyperion. Thomas *et al.* estimate a rotational period of 13 days (*Nature* 307, 716; 1984), which, since the orbital period is 21 days, tends to confirm that Hyperion has yet to reach synchronous rotation. They also claim, however, that Voyager 2 images show a spin which is coherent over 61 days and that the derived spin period is consistent between the Voyager 1 and Voyager 2 photographs, even though the sequences were made 220 days apart. If Hyperion has maintained the same spin period for this length of time then the balance swings in favour of ordered rather than chaotic

motion. Wisdom *et al.* argue, however, that if the spin is chaotic, with variations over a few orbital periods, then the standard technique of fitting observations made at different times to a constant period is not applicable. The conflicting evidence can only be resolved by an accurate determination of Hyperion's light curve.

Wisdom *et al.* give a convincing demonstration of how some of the techniques of modern dynamics can be applied to the Solar System. It remains to be seen whether or not the 'majestic clockwork' of Kepler and Newton has an unpredictable component. □

Carl D. Murray is in the Theoretical Astronomy Unit, School of Mathematical Sciences, Queen Mary College, Mile End Road, London E1 4NS.

Plant engineering

More prospects for Ti plasmids

from M.H. Drummond

AGROBACTERIUM TUMEFACIENS causes crown gall tumours in some gymnosperms and most dicotyledonous angiosperms¹ by inserting its Ti plasmids into the nuclear genome of the infected plant; and agrobacterial Ti plasmids are now well established as vectors for introducing foreign genetic material into a wide range of higher plants^{2,3}. This may ultimately make possible useful additions to the genomes of crop plants. Unfortunately the prospect for using Ti-based vectors in monocotyledonous plants has been gloomy because *A. tumefaciens* is not a pathogen for them, a matter of considerable regret since the monocots include the grass crops, which feed the greater part of mankind. On page 763 of this issue, however, Hooykaas-Van Slooter *et al.* indicate that Ti plasmid DNA enters the cells of at least some monocot species, but is simply not oncogenic⁴. Vectors based on Ti plasmids may thus be useful in monocots after all.

The observation made by the Dutch group concerns the presence in plant tissue of opines. These metabolites are absent from normal tissue but are synthesised in large amounts in crown gall tissue by synthases encoded in T-DNA, the Ti plasmid segment that is integrated into the plant genome. The best known opines, octopine and nopaline, characterize the two principal varieties of Ti plasmid. Ti plasmid sequences outside the T-DNA segment encode oxidases that catabolize opines in the bacterium. Thus the plasmid both diverts the biosynthetic resources of the plant and enables its bacterial carrier to exploit the resulting opine pool as a very specific carbon and nitrogen source. This strategem has been termed genetic colonization. The ecological niche it creates is further enlarged by multiplication of opine-producing cells into a crown gall tumour. This is brought about by other T-DNA genes which perturb hormone metabolism in the plant cell, raising the level of both auxins and cytokinins⁵. The Ti plasmid vectors developed for genetic engineering can be 'disarmed' by deleting, or otherwise mutating, their oncogenic genes, in order to permit differentiation of engineered tissue into entire plants^{3,6}.

The Dutch group find that when certain ornamental monocots are infected with virulent strains of *A. tumefaciens*, opine synthesis ensues in the absence of any sustained neoplastic growth. Opine synthase genes are known to be under the control of eukaryotic regulatory sequences⁷. The remote possibility that they are being expressed by the bacterial cell in this system is excluded by the observation that no octopine synthesis is detectable when T-DNA transfer is blocked by a plasmid mutation,

virB, outside the T-DNA⁸. The obvious interpretation is that T-DNA does enter the monocot cell, but fails to bring about oncogenesis. If so, Ti plasmids, far from being unsuitable for the genetic engineering of monocots, can be considered well-suited because already disarmed, although it remains to be seen whether the Dutch group's observations can be extended to the important grass crops.

The definitive experiment, demonstrating the presence of T-DNA sequences in monocot tissues by nucleic acid hybridization, remains to be carried out. Southern blotting is now used routinely to characterize DNA sequences inserted into dicot genomes^{2,3,9}, but this is more problematical for monocots because of difficulties in the culture of large amounts of transformed tissue without significant contamination by normal cells. In dicots the hormone autotrophy conferred by T-DNA facilitates the selection and growth of transformed tissue, and in many species the establishment of tissue culture lines from single cells is quite straightforward. This is not yet the case for monocots but other means of deriving monocot tissue enriched for cells containing inserted DNA should be feasible. For example, a Ti vector encoding neomycin phosphotransferase such as that constructed by Bevan *et al.*⁹ could be used in combination with G418 drug selection in tissue culture. Neomycin resistance is expressed from the *nos* (nopaline synthase) promoter in this construct, which underlines the importance for

monocot genetic engineering of the present demonstration that synthase genes are transcribed in the monocot genetic background.

If T-DNA is integrated into the monocot genome in its entirety, its failure to induce tumorigenesis must be accounted for. There are two obvious possibilities: either the oncogenic genes are not properly expressed, or their protein products fail to disrupt control of cell division. If the oncogenic genes are not transcribed, then comparing their regulatory regions with the *nos* or *ocs* (octopine synthase) promoters might bring to light differences between monocot and dicot promoter sequences. However, the Dutch group seem to favour the second possibility.

The work has one further interesting aspect. By illustrating nicely that oncogenesis is not an essential part of genetic colonization, it raises the question of whether other procaryotes have evolved similar stratagems for exploiting plant or animal hosts. To discover any that were as useful as Ti plasmids would be very exciting, but in the absence of any obvious pathogenic effect, how does one start looking? □

1. Elliot, C. *Manual of Bacterial Plant Pathogens* 2nd edn (Chronica Botanica, Waltham, 1951).
2. Herrera-Estrella, L. *et al.* *Nature* **310**, 115 (1984).
3. Barton, K.A. *et al.* *Cell* **32**, 1033 (1983).
4. Hooykaas-Van Slooter, G.M.S., Hooykaas, P.J.J. & Schilperoort, R.A. *Nature* **311**, 763 (1984).
5. Akiyoshi, D.E. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 407 (1983).
6. Zambryski, P. *et al.* *EMBO J.* **2**, 2143 (1983).
7. De Greve, H. *et al.* *J. molec. appl. Genet.* **1**, 499 (1983).
8. Hoekema, A. *et al.* *Nature* **303**, 171 (1983).
9. Bevan, M.W., Flavell, R.B. & Chilton, M.D. *Nature* **304**, 184 (1983).

M.H. Drummond is in the Agricultural and Food Research Council Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ.

DNA recombination

Resolution of intermediates

from Nancy L. Craig

HOLLIDAY structures¹ are thought to join DNA duplexes by a reciprocal single-strand exchange and are thought to be intermediates in various pathways of genetic recombination (Fig. 1). They have been visualized by electron microscopy, but biochemical evidence in support of their role in recombination has been hard to come by. Now, proteins encoded by *Escherichia coli* phages are beginning to provide information about breakage and rejoining reactions that can mediate the formation and resolution of Holliday structures. Kemper *et al.*² and de Massy *et al.*³ have characterized phage endonucleases that can specifically cleave Holliday structures *in vitro*, probably reflecting an important step in the resolution of Holliday intermediates in homologous recombination. And on page 721 of this issue, Hsu

and Landy show that Holliday structures can be resolved *in vitro* to recombinant duplexes by the site-specific recombination machinery of phage lambda, providing strong evidence that Holliday structures are intermediates in this recombination pathway⁴.

Integration of phage lambda into the *E. coli* chromosome occurs by reciprocal combination between *attP*(POP'), a ~240-base pair (bp) specific site in the phage chromosome, and *attB*(BOB'), a ~25-bp site in the bacterial chromosome⁵ (see Fig. 2). The products of integrative recombination are the hybrid sites *attL*(BOP') and *attR*(POB') which flank the integrated prophage. Prophage excision occurs by reciprocal recombination between *attL* and *attR*, regenerating *attP* and *attB*. Each *att* site contains the same

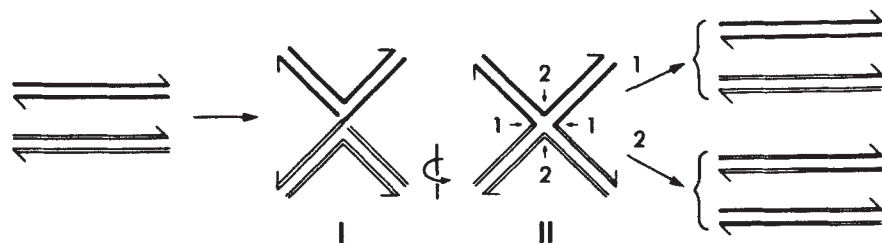


Fig. 1 Formation and resolution of a Holliday structure. The polarity of the strands in two DNA duplexes is indicated by the arrowheads. A Holliday structure (I) is formed by breakage, exchange and rejoining of a pair of strands of like polarity at equivalent positions. Rotation produces II, an isomer of I. A Holliday structure is resolved by breakage, exchange and rejoining of a pair of strands of like polarity at the branch point; the outcome depends on whether this occurs at positions 1 or 2.

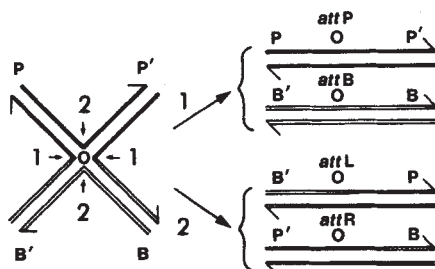


Fig. 2 Resolution of a Holliday intermediate in lambda site-specific recombination. A Holliday structure containing *att* sites is shown. P, P', B, and B' are the main *att* sites. The branch point occurs in the core (O), a sequence common to all *att* sites. Resolution at positions 1 yields the products of excisive recombination, *attP*(POP') and *attB*(BOB'). Resolution at positions 2 yields the products of integrative recombination, *attL*(BOP') and *attR*(POB').

15-bp core (O), flanked by unique arms (P, P', B, B'); see Fig. 2. Integrative recombination requires Int, a lambda-encoded protein, and integration host factor (IHF), a host-encoded protein. Excisive recombination requires Int, IHF and Xis, another phage-encoded protein. These proteins are specific DNA-binding proteins and the *att* sites contain elaborate arrays of recognition sequences.

Using a method developed by Bell and Byers⁶, Hsu and Landy⁴ constructed pairs of *att* site-containing DNA duplexes with four non-homologous arms (Fig. 2) joined by a reciprocal single-strand exchange in the core. Incubation of these structures with purified Int resulted in their resolution to the products of excisive and integrative recombination. Both pairs of recombinants are observed because resolution can occur either at positions 1 or positions 2 of the Holliday structure (Fig. 2). These experiments suggest that natural Int-dependent recombination proceeds via the formation of a Holliday structure by a reciprocal single-strand exchange between the cores of *att* sites, followed by its resolution by a second reciprocal exchange.

The demonstration that Int alone can promote resolution emphasizes its central role in strand exchange and extends the finding that Int has specific topoisomerase activity which breaks and rejoins DNA at the junction-type Int recognition sequences in the cores of *att* sites⁷. Hsu and Landy show that resolution, like recombi-

nation, proceeds in the absence of a high energy cofactor and produces intact duplexes, features reflecting the action of a topoisomerase. Moreover, they also found that Int's capacity for resolution is specific to Holliday structures containing *att* site cores at the branch point. Int can resolve synthetic Holliday structures that lack distal segments of the arms of the *att* sites but does not resolve synthetic Holliday structures which lack the core. Thus, it is likely that Int topoisomerase mediates strand exchange by promoting sequence-specific breakage and rejoining reactions in the cores of *att* sites and that these reactions form and resolve Holliday structures. The action of topoisomerases may also underlie other site-specific recombination reactions^{8,9}.

What are the roles of the other components of the lambda site-specific recombination machinery? Hsu and Landy show that sequences in the *att* site arms which are essential for recombination are not required for resolution. This suggests that the interactions of IHF, Xis and Int with their recognition sequences in the arms of *att* sites are important in promoting the formation of a Holliday structure. The formation of such an intermediate is a multi-step reaction in which recognition of the *att* sites by the recombination proteins and synapsis of the *att* sites precede the strand-exchange step, which is mediated by the interaction of Int with the core. The assembly of the *att* sites and recombination proteins into organized complexes¹⁰ may facilitate these steps.

Hsu and Landy have also observed that, although an *att* site-containing a Holliday structure can be resolved by Int alone to the products of either integrative or excisive recombination, the relative efficiencies of these two reactions vary under different experimental conditions. This suggests that resolution may also be regulated by other components of the recombination machinery.

Formation and resolution of Holliday structures is also probably involved in strand exchange in homologous recombination. Study of the *E. coli recA* protein is revealing how Holliday structures can be formed during homologous recombination¹¹. How can they be resolved? Purified endonuclease VII (endo VII), an enzyme encoded or regulated by coliphage T4 gene 49, cleaves Holliday structures *in*

vitro by breaking pairs of DNA strands of like polarity at the branch point — that is, at positions 1 or positions 2 (see ref. 12). De Massy *et al.*³ have now shown that phage T7 endonuclease I, which is the gene 3 product, has similar activity. It has now been shown that endo VII (refs 2 and 13) and endo I (ref. 3) cleave at the branch points of cruciforms which occur at short inverted repeats in supercoiled DNA. This result suggests that such cruciforms are useful analogues of Holliday structures and that these enzymes will also be useful as probes of cruciform structure.

The resolution of Holliday structures promoted by endo VII and endo I is significantly different from Int-promoted resolution. In contrast to Int, whose resolving capacity is limited to *att* site-containing Holliday structures, endo VII and endo I are capable of resolving Holliday structures that contain a variety of sequences, although endo VII has a modest sequence preference². Furthermore, in contrast to Int, neither endonuclease promotes rejoining of the DNA strands they break. Thus, in homologous recombinations of bacteriophage DNA, resolution of Holliday intermediates to intact recombinant duplexes probably involves the action of other enzymes, for example, DNA ligase. It is also likely that endo VII and endo I participate only in the resolution step in recombination, whereas Int mediates both the formation and resolution of recombination intermediates.

Hsu and Landy provide the first physical evidence that Holliday structures are intermediates in phage lambda site-specific recombination. It will now be possible to dissect separately the formation and resolution of these intermediates. Although further work is needed to establish that endo VII and endo I participate directly in homologous recombination between phage DNA molecules *in vivo*, detection of activities that can resolve Holliday structures supports the hypothesis that these structures are recombination intermediates. Using the methods developed to detect the activity of endo VII and endo I towards Holliday structures, it should be possible to search for similar cellular activities. □

- Holliday, R. *Genet. Res.* **5**, 282 (1964).
- Kemper, B., *et al.* *Cold Spring Harb. Symp. quant. Biol.* **49** (in the press).
- de Massy B., *et al.* *Cold Spring Harb. Symp. quant. Biol.* **49** (in the press).
- Hsu P.L. & Landy, A. *Nature* **311**, 721 (1984).
- Weisberg, R. & Landy, A. in *Lambda II* (eds Hendrix, R., Roberts, J., Stahl, F. & Weisberg, R.) 211 (Cold Spring Harbor Laboratory, New York, 1983).
- Bell, L. & Byers, B. *Proc. natn. Acad. Sci.* **76**, 3445 (1979).
- Craig, N. & Nash, H. *Cell* **35**, 795 (1983).
- Abremski, K., Hoess, R. & Sternberg, N. *Cell* **32**, 1301 (1983).
- Krasnow, M. & Cozzarelli, N. *Cell* **32**, 1313 (1983).
- Better, M., Chi, L., Williams, R. & Echols, H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5837 (1982).
- Radding, C. A. *Rev. Genet.* **16**, 405 (1982).
- Mizuuchi, K., Kemper, B., Hays, J. & Weisberg, R. *Cell* **29**, 357 (1982).
- Lilley, D. & Kemper, B. *Cell* **36**, 413 (1984).

Nancy L. Craig is in the Department of Microbiology and Immunology, School of Medicine, University of California, San Francisco, California 94143.

Meteorology

Origins of stony-iron meteorites

from Edward R.D. Scott

ALTHOUGH most of the meteorites that fall on Earth are chondrites, more is known about the formation and parent bodies of the 15 per cent that come from bodies that were once largely molten. Three such igneous meteorites come from the surface of the Moon¹, eight may have come from Mars², and a large group — known as basaltic achondrites — may be pieces of 4 Vesta, the second or third largest asteroid^{3,4}. But what is the origin of the variety of igneous meteorites known as stony-irons, which account for only seven per cent of falls of igneous meteorites but whose unusual mineralogy constitutes a major puzzle? New astronomical and theoretical studies indicate how and where the stony-iron meteorites may have formed in the asteroid belt.

Most igneous meteorites contain either less than 1% or greater than 99%, by weight, of metallic iron-nickel and other metallic phases. This is because the large density difference between molten iron and silicate ensures their rapid separation. By contrast, stony-irons usually contain 40–60 wt % iron-nickel (and always 20–80 wt %). In pallasites, the remainder is olivine ($(\text{Mg}, \text{Fe})_2\text{SiO}_4$), whereas in mesosiderites it is basaltic material, which lacks significant amounts of olivine.

How were large volumes of metal intimately mixed with massive amounts of mantle olivine or crustal basalts? For the pallasites, it is widely believed that metal from the core of a molten asteroid was forced into part of the surrounding olivine mantle as a result of cooling stresses, impacts and the weight of the mantle. But it is much more difficult to understand how mesosiderites formed. Petrological studies show that brecciated basaltic material from the surface of an asteroid was mixed with metallic iron-nickel without the inclusion of significant quantities of mantle olivine. At 1,000°C, the cooling rate of the mixture was about one degree per year but because the mesosiderites were eventually buried so deeply, their rate of cooling at 500°C was reduced by at least 10⁵-fold and so was slower than that of any other type of meteorite.

Conventional models for the origin of mesosiderites attribute the mixing of metal and silicate to impacts between two igneously differentiated asteroids, and the slow cooling to subsequent accretion. However, R. Greenberg and C.R. Chapman⁵ argue that the lack of olivine, the uniform cooling rates and the abundance of mesosiderites preclude such models and suggest instead that the mixing occurred within a single asteroid. In their scheme, a solid basaltic crust of a largely molten body sank towards the core-mantle interface

when it was fractured by impacts because it was slightly denser than a liquid mantle beneath it. Liquid iron from the core penetrated the cold fractured blocks of crust so that the mixture cooled rapidly at high temperatures but much more slowly at low temperatures. Greenberg and Chapman suggest that pallasites formed in smaller bodies in which the olivine-rich mantles solidified before the crusts foundered.

Reviewing this and other proposed origins for mesosiderites, R. Hewins⁶ concludes that no single model is wholly satisfactory. He finds that mesosiderite textures resemble those of rocks formed by impacts and believes that major collisions must have mixed metal and silicate. Hewins suggests that mesosiderites lack olivine because, unlike all known oxidized chondrites, their parent body never contained much olivine — an argument not favored by Greenberg and Chapman.

There has been no certain identification of asteroids with mesosiderite-like surfaces, although several S-type asteroids have been proposed as candidates. However, recent telescopic observations have revealed the existence of a new class of asteroids with surfaces that resemble those

of pallasites. According both to D.P. Cruikshank and W.K. Hartmann⁷ and to J.F. Bell and coworkers⁸, infrared spectra of 246 Asporina, 289 Nenetta and 446 Aeternitas show the deep absorption bands of olivine and overall red colors that are consistent with the presence of a metal phase. Hence some asteroids of 50–100 km diameter have melted to form olivine-rich mantles that have since been exposed by collisional stripping of the crusts.

To learn more about the origins of mesosiderites and pallasites we must reach a much better understanding of the nature and distribution of the heat source, and of the movement of solid and liquid phases during melting and solidification. We need to know whether totally molten mantles of olivine-rich composition could be produced, and whether melting occurred after or during accretion in the asteroid belt. Theory and experiment will help to address these questions but to understand fully the origins of stony-irons we shall also need field studies of asteroids. □

1. Eberhardt, J. *Science News* **126**, 70 (1984).
2. McSween, H.Y. Jr. *Geology* **12**, 3 (1984).
3. Drake, M.J. in *Asteroids* (ed. Gehrels, T.) 765 (University of Arizona, 1979).
4. Chapman, C.R. *Planetary Report* **4**, 12 (1984).
5. Greenberg, R. & Chapman, C.R. *Icarus* **57**, 267 (1984).
6. Hewins, R.H. *J. geophys. Res. Suppl.* **88**, B257 (1983).
7. Cruikshank, D.P. & Hartmann, W.K. *Science* **223**, 281 (1984).
8. Bell, J.F., Hawke, B.R., Singer, R.B. & Gaffey, M.J. in *Lunar and Planetary Science XV*, 48 (Lunar and Planetary Institute, Houston 1984).

Edward R.D. Scott is in the Institute of Meteoritics, University of New Mexico, Albuquerque, New Mexico 87131.

Shiro Kakiuchi 1929–1984

SHIRO Kakiuchi of Osaka University Medical School, who died on 22 September, was a pioneer in many areas of cell regulation. During the mid 1960s he worked with T. E. Rall at Case Western Reserve in Ohio and made many contributions to cyclic nucleotide research during the formative years of this field. It was during this time that he began work on cyclic nucleotide phosphodiesterases. In 1970, soon after his return to Japan, Dr Kakiuchi revealed that a form of phosphodiesterase required the presence of an activator and that stimulation by the activator required Ca^{2+} -dependent protein activator that later came to be called calmodulin.

Kakiuchi realized the potential importance of the activator in other enzyme systems and in 1978 recognized that it was also the activator of myosin light chain kinases. Subsequently he isolated the protein from a variety of ciliates and invertebrates and discovered a calmodulin-dependent form of guanylate cyclase. In the same year, Kakiuchi found that calmodulin was partitioned between the soluble and particulate fractions of the cell, which led him to the discovery in 1980 that the only calmodulin-binding protein in the cytoskeleton of the red blood cell is spectrin. A year later he was the first to show the

presence of a spectrin-like protein in other tissues and to purify the protein from brain. Also in 1981 he purified another calmodulin-binding protein from chicken smooth muscle and showed that it interacted with actin filaments.

This protein, which he called caldesmon, was subsequently named fodrin by US scientists. Caldesmon interacts with actin when the Ca^{2+} concentration is less than 1 μM but with calmodulin at higher concentrations. This led to the hypothesis that calmodulin might control those proteins associated with actin in a 'flip-flop' mechanism depending on the Ca^{2+} concentration. The hypothesis was later extended to the Ca^{2+} regulation of microtubules when Kakiuchi showed that one of the microtubule-associated proteins, *tau*, was a Ca^{2+} -dependent calmodulin binding protein.

All of these experiments were pioneering efforts and each observation has been repeated and extended by numerous laboratories. As much as any one individual Shiro Kakiuchi was the father of the field of calmodulin research and an innovator in the study of its interaction with other regulatory proteins. He will be passionately missed by his many students, collaborators and friends. **A.R. Means**

Geomagnetic secular variation impulses

V. Courtillot & J. L. Le Mouél

Laboratoire de Géomagnétisme Interne et Paléomagnétisme (ERA CNRS 922), Institut de Physique du Globe, Université Paris 6 et UER Sciences Physiques de la Terre, Université Paris 7, 4 Place Jussieu 75230 Paris Cedex 05, France

The secular variation of the Earth's internal magnetic field changes on time scales of the order of a year. Such impulses occurred in 1969 and probably in 1913; they provide constraints on deep mantle conductivity, which appears to be rather low, and on the motions of the top of the fluid core, which are probably dominated by westward drift. Present data favour the existence of upwelling of core material at the core-mantle boundary. The impulses can be correlated with extrema in the Earth's rotation rate and interpreted in terms of electromagnetic core-mantle coupling.

THE study of temporal variations of the Earth's magnetic field has benefitted from observational advances in the past 5 yr. Secular variation of the geomagnetic field observed at the Earth's surface has been found to undergo rapid accelerations lasting less than a few years, a much shorter duration than had previously been recognized for signals generated in the Earth's core and having diffused through the electrically-conducting mantle. One such rapid and worldwide acceleration occurred in 1969; an earlier, less well documented, acceleration apparently occurred around 1913. The identification of these events was made possible by carefully assembled long data sets from magnetic observatories around the world. The centenary of the French Magnetic Observatory (St Maur-Val Joyeux-Chambon La Forêt) provided the opportunity for a meeting to discuss recent progress on the origin and secular variation of the geomagnetic field. We now review evidence (in part presented at the meeting) for the occurrence of impulsive accelerations in geomagnetic secular variation. Also discussed are the implications of these events regarding deep mantle conductivity, their link with decade fluctuations in the rotation of the Earth, the case for core-mantle coupling and efforts to determine core motions.

Secular acceleration impulse of 1969

Short-period variations of the geomagnetic field are linked with electric currents flowing in the ionosphere and magnetosphere (thus having a primarily external origin), whereas longer-period variations are thought to be due to dynamo processes acting within the Earth's fluid metallic core. It was accepted until recently that the cutoff between the two was about 4 yr (see ref. 1). This cutoff was raised to more than 10 yr by Currie² and Alldredge³, although it was recognized that signals with a clear external origin, such as the 11-yr solar cycle, fell close to this limit (see ref. 4). In a previous study⁵ of observatory data for the period 1947–1972, we found no evidence for short-period signals that would have originated from the core. However, when attempting to separate the solar cycle from long-period internal variations, we noticed discrepancies in the correlation between variations seen on the horizontal versus vertical components, particularly between 1967 and 1972 (the date of the most recent data then available). These suggested that the effect could be due to a slight change in the second derivative of the 'true' internal secular variation. With data available up to 1977, it has been shown⁶ that there had been an impulse in the third derivative of geomagnetic field components around 1969. The impulse was particularly spectacular in the east (Y) component of European observatories and was identified in at least 38 observatories from the Northern Hemisphere (Fig. 1).

Extremal secular variation behaviour had already been observed by Vestine⁷ to have occurred around 1910. The concept of secular variation impulses was introduced by Walker and O'Dea⁸, who found it convenient, for the purpose of correcting magnetic data to a given epoch, to approximate the secular variation by a constant value for a chosen interval. In this

process, changes in constants from one interval to the next resulted in 'impulses'. These impulses were formerly attributed to the internal field (see refs 9, 10) but Alldredge¹¹ showed that the impulses were correlated with the sunspot cycle and resulted from an ill-chosen piecewise linear fit to the smooth external variations. We confirmed⁵ that the slope breaks resulting in impulses coincided with extrema in the 11-yr variation.

The 1969 impulse is a completely different matter both in time evolution, geographical distribution and intensity, being an order of magnitude larger than the constructions of Walker and O'Dea. The original⁶ presentation of the impulse was based on a representation of the field components themselves, which were interpreted in terms of two parabolic segments, with discontinuous curvature (that is second derivative) in 1969. This meant that the third derivative of the field components could be represented by a Dirac distribution. The parabolic representation was criticized by Alldredge¹² who suggested that the impulse might be an artefact of the fitting procedure. In response to this comment, Courtillot *et al.*¹³ showed that displaying the data as first derivatives (computed simply from first-order finite differences) provided a very clear and convincing picture of the impulse (Fig. 1).

Parallel to the work done in Paris, a team led by Malin in Edinburgh observed, when preparing the 1975 World Magnetic Charts, the sudden change in secular acceleration of declination which occurred in 1969 in European observatories: Malin *et al.*¹⁴ showed that the representation of secular variation as a function of time was to be preferred over that of the original data or of its second derivative. In the case of second-order finite differences, higher frequency noise is amplified together with harmonics of the solar cycle, in particular the harmonic with a period of 3.7 yr. Careful filtering can, however, reveal the step change in acceleration at the expense of time resolution (J. G. Gavoret, personal communication). Thus, together with Courtillot *et al.*¹³, Malin *et al.*¹⁴ concluded that data are well represented by a simple instantaneous change of slope between two linear segments and that the representation as "the sum of a series of sine curves of different frequency", proposed by Alldredge¹², "is an unnecessarily complicated and conceptually unhelpful way of regarding the phenomenon".

Working on first order differences for the X(north), Y and Z(vertical) annual mean values from 130 observatories, Chau *et al.*¹⁵ and Le Mouél *et al.*¹⁶ were able to confirm the worldwide and simultaneous character of the 1969 impulse. The amplitude of the step in the second time derivative was estimated from a least-squares fit of two linear segments to the data. The date of the impulse was determined by minimizing the r.m.s. residuals for impulse dates ranging from 1967 to 1972, and was found to be 1969. The worldwide distribution of impulse amplitude for each field component is shown in Fig. 2. Departure from the V shape in original observatory data may be due to remaining external signals, to problems in baseline control (up to 1970 most frequently in the Z component) and to more localized

internal signals with a different behaviour, as is apparently the case under Central America and the Indian peninsula. In any case, the resulting picture is smooth and coherent.

Malin *et al.*¹⁴ analysed the D (declination), H (horizontal intensity) and Z annual mean values for 184 worldwide observatories. They assumed that the magnetic field associated with the impulse was internal and performed a spherical harmonic analysis on 3-yr smoothed means, limiting the expansion to degree and order 6. Secular variation models were derived (by subtracting) for epochs 1963.5, 1965.5 and 1967.5, and for epochs 1971.5, 1973.5 and 1975.5, providing two estimates of secular acceleration, one centred on 1965.5, the other on 1973.5. Accelerations were derived by least-square fits of linear time functions to the secular variations, both before and after the impulse, and the resulting impulse amplitudes (1973.5 minus 1965.5 accelerations) truncated to degree and order 4 were given by Malin *et al.*¹⁴. The maps which can be derived from this truncated representation are in reasonable agreement with those of Le Mouél *et al.*¹⁶, although differences exist. The largest amount of power in the Malin *et al.*¹⁴ expansion is in the $n=2$ terms. In a more recent analysis, Malin and Hodder¹⁷ resumed the experiment for the components from 83 selected observatories, this time including the possibility for an external part in the field. The expansion was limited to degree and order 4. They found that most of the jerk is indeed of internal origin.

Inspection of the data from ref. 16 (see Fig. 2) reveals wide areas where the distribution of impulse elements are rather flat (such as central Asia for δX and δZ , or South America for δY) separated by narrow areas with steep gradients where sign changes occur (such as in northern Central America or South East Africa for δZ). Thus, an accurate description of the impulse distribution may require high-order spherical harmonics or a different representation; available data do not allow such a detailed expansion. However, the simplicity of the overall pattern demonstrated by the few and smooth zero-isolines of Fig. 2 favours the dominance of low (particularly $n=2$) order terms^{14,18}.

Secular acceleration impulse of 1913

Following the identification of the 1969 impulse, it was natural to search for earlier occurrences of such a phenomenon that might have escaped identification. Vestine⁷, for example, had noticed large changes in secular variation around 1910, and we noticed a sharp change in the secular acceleration of declination shortly after the beginning of this century at Val Joyeux ("Chambon"). Courtillot *et al.*⁶ tentatively identify this event on the Y component of many observatories. Ducruix *et al.*¹⁹ analysed annual mean values of X , Y and Z from 40 observatories in the Northern Hemisphere and 10 observatories in the Southern Hemisphere. They observed that for many observatories and many components secular variation roughly follows a linear trend between 1913 and 1969, which is preceded by a global break in the secular variation slope occurring around 1913 (Fig. 1). In certain areas, other internal variations of regional importance distort the simple piecewise linear (V) shape of the secular variation. Such is the case for the Y component in European observatories where an anomaly centred on 1925 renders the identification of the 1913 impulse more difficult. Ducruix *et al.*¹⁹ outlined the zero isolines of the 1913 impulse for the three components. The geometry of the impulse is reminiscent of that for the 1969 impulse but with a reversal in sign. Secular variation may also have been linear between 1905 and 1913, but data for the early years of the century and last years of the previous century are too scarce, and sometimes too noisy, to allow a satisfactory global analysis of the event. The identification of impulses before 1910 is difficult. Only 19 observatories were in operation in 1900, 12 of them in western European countries. However, long homogeneous series of data such as that provided by Malin and Bullard²⁰ for London or that being prepared for Paris (J.L. Le M. *et al.*, in preparation) may help in searching for earlier events.

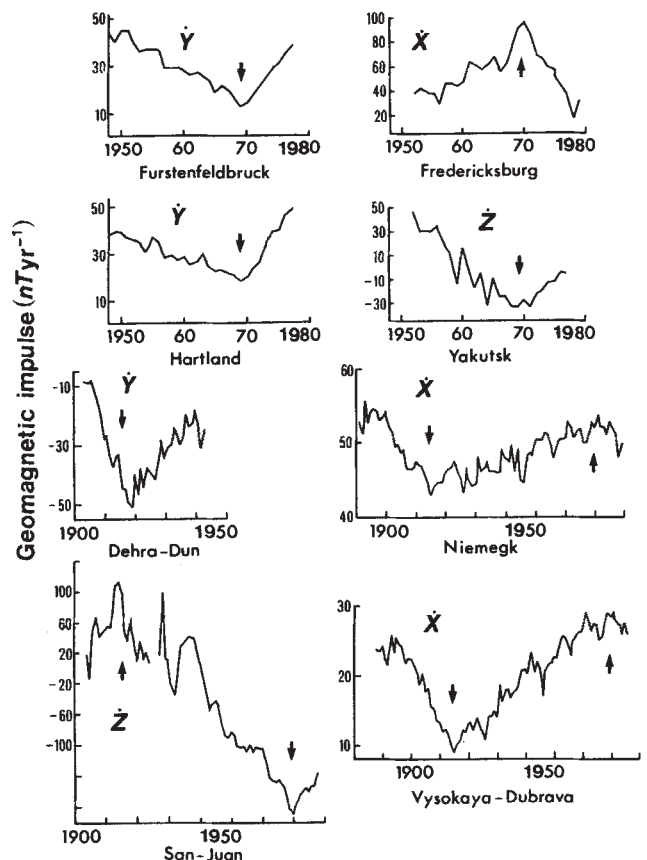


Fig. 1 Secular variation (first difference of annual mean values) of geomagnetic components X (north), Y (east) and Z (vertical) in widely-spaced observatories (from refs 13, 16 and 19). Furstenfeldbruck, Hartland and Niamegk are in Europe, Fredericksburg in the USA, Yakutsk and Vysokaya-Dubrava in the USSR, Dehra-Dun in India and San Juan in Puerto-Rico. The occurrences of the ≈ 1969 and ≈ 1913 impulses are outlined by arrows.

Secular variation 1910–80

Ducruix *et al.*¹⁹ and Gire *et al.*¹⁸ conclude that the secular variation field can be modelled in a remarkably simple way from at least 1913; as a first approximation, each component of the secular variation can be represented as a two-segment broken line, with an apparently worldwide and synchronous break occurring in 1969 and probably an earlier one in 1913 (Fig. 3).

Using spherical harmonic coefficients provided by Hodder²¹, Gire *et al.*¹⁸ found that, between 1913 and 1969, the secular variation isovalue lines became wider apart and that the energy of secular variation was reduced by a factor 2. Between 1969 and 1980 the secular variation field recovered its strength and in 1980 the maps displayed strong similarities to those in 1912, except in the Far East where data are scarce. The linear model proposed by Gire *et al.*¹⁸ accounts for more than 75% (in terms of energy) of the total secular variation observed for the X and Y components. Early values for the Z component are inaccurate and the piecewise linear model does not work as well.

Mantle conductivity

The classical way of probing the electrical conductivity of the Earth's mantle is to study transient geomagnetic variations of primary external origin and to separate the internal and external parts of the signal as it is observed at the Earth's surface (see ref. 22). The longer the period of the signal, the deeper its penetration depth. The identification of the solar-cycle variation and the separation of its internal and external parts have yielded some of the deepest estimates of mantle conductivity (down to $\sim 1,500$ km; refs 3–5, 23–28).

Another approach of the mantle conductivity problem uses the diffusion of magnetic signals generated in the Earth's core.



© 1984 Nature Publishing Group

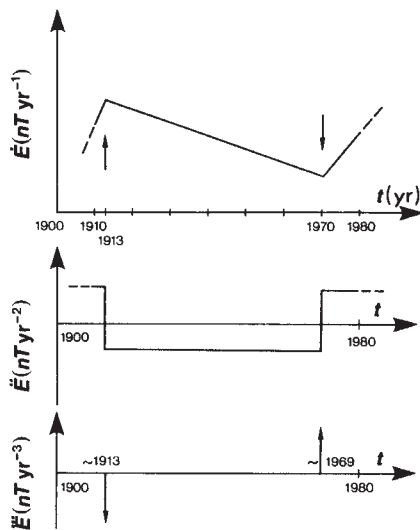


Fig. 3 A simple secular variation (first-time derivative) model for any geomagnetic element E involving two impulses in 1913 and 1969 and linear variations between the impulses. The second and third derivatives of E are also sketched.

Runcorn⁹ used the "secular variation impulses" introduced by Walker and O'Dea⁸ to infer an upper bound of the mean mantle conductivity, which he found to be of the order of $10^2 \Omega^{-1} \text{m}^{-1}$. McDonald¹⁰ further studied the penetration of secular variation, including the 'impulses', through the mantle and derived a profile for the conductivity of the entire mantle, rising slowly from $30 \Omega^{-1} \text{m}^{-1}$ at 1,200 km depth to $200 \Omega^{-1} \text{m}^{-1}$ at the core-mantle interface. However, note that the impulses introduced by Walker and O'Dea are artefacts. Following the positive identification of the 1969 impulse, Achache *et al.*^{29,30} resumed Runcorn's early analysis. These authors erroneously modelled the 1969 event as a second-order impulse, that is a step change in secular variation, and not acceleration³¹. The shape of the secular variation curves after 1969 was interpreted as part of the transient impulse response leading to a mean conductivity for the lower 2,000 km of the mantle of the order of $150 \Omega^{-1} \text{m}^{-1}$. This result was included in the global study of Achache *et al.*²⁸, who proposed a conductivity of the order of $300 \Omega^{-1} \text{m}^{-1}$ in the lower 700 km of the mantle.

Backus³¹ has shown that when the radial component of the time-dependent magnetic field measured at the Earth surface is expanded in surface spherical harmonics, each spherical harmonic component of a given degree l observed at the surface is related to the same component at the Earth's core by a causal linear filter. Backus showed that the filter impulse response can formally be treated as a probability density function, whose mean τ_l and standard deviation σ_l are the delay time and smoothing time of the filter: the main effects of the l th mantle filter are to delay the input by τ_l yr and to smooth it in a gaussian way over σ_l yr. In principle, τ_l and σ_l can be evaluated from observations through spherical harmonic analysis, and thus they provide functions of (constraints on) mantle conductivity. Backus provided the way to apply mantle filter theory to an impulse of order p , that is, one for which the p th derivatives of geomagnetic components are Dirac distributions: if the impulse starts at the core surface at time t_0 and has an amplitude A_p , one can derive from the data the order p of the impulse, A_p , σ_l and $t_0 + \tau_l$, but not t_0 and τ_l separately. Moreover, Backus stresses that each component of the field at a single location is a linear combination of the outputs for all degrees l and that if this composite signal is processed as if it were the output of a single filter, the resulting fictitious mixed filter can have a negative delay time τ_l and imaginary smoothing time σ_l . Applying the theory to the 1969 impulse as observed in Europe on the Y component (that is, the record on which the impulse was discovered and is particularly clear), Backus finds that $t_0 + \tau = 1969.8 \pm 0.2$ (corrected from the original paper to account for

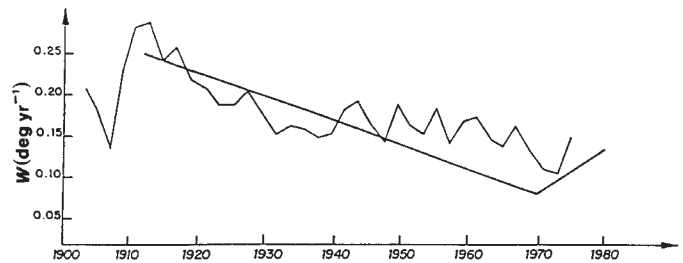


Fig. 4 Estimates of the westward drift rate computed for the period 1910-80 from a V-shaped model (straight lines, after refs 19 and 34) and using Hodder's^{20,36} results (curved line).

the fact that annual mean values are centred on 1 July), $p = 3$, $A = 5.0 \pm 0.3 \text{ nT yr}^{-2}$ and $\sigma^2 = -2 \pm 1 \text{ yr}$. Backus³¹ concludes that harmonic mode mixing in Europe is probably responsible for the observed negative value of σ^2 (although a sunspot-related contribution or other source of noise remains a possibility). Even in the case where mode mixing does not occur, Backus remarks that the 1969 impulse permits values of σ as high as 2 yr with harmonics as high as order 4 and concludes that this is compatible with a lower mantle conductivity as high as several thousands of $\Omega^{-1} \text{m}^{-1}$.

Courtillot *et al.*³² elaborated on Backus's³¹ results and emphasized the fact that a sunspot-related signal could be responsible for the negative value of σ^2 . They generated a synthetic curve, adding a typical sunspot signal to a pure third-order impulse, and found that the short data window available after the impulse indeed generated a sunspot related bias of σ^2 , with a magnitude close to the observed one. Courtillot *et al.*³² argued that the 1969 impulse is apparently worldwide and synchronous and that in many observatories, for many components, the transition from the pre-impulse to the post-impulse 'asymptotic' behaviour seems to take on the order of 1 yr. This led Courtillot *et al.*³² to propose that harmonic mode mixing could be of minor importance.

Backus³¹ showed that, for reasonable conductivity distributions, the ratio of smoothing time to delay time of a mantle filter has to lie between 0.3 and 0.8. Courtillot *et al.*³² applied this constraint and pointed out that if σ , as they believe, is of the order of 1 yr, then τ must be $< 3 \text{ yr}$. Conversely, if the τ_l are indeed 13 yr, σ_l must be $> 4 \text{ yr}$ (ref. 31 and G. Backus personal communication). Available data should allow a discrimination between $\sigma_l \leq 1 \text{ yr}$ and $\sigma_l \geq 4 \text{ yr}$.

Using their preferred values of σ and the dominating spherical harmonic order $l=2$ of Malin and Hodder¹⁷, together with Backus's³¹ formalism, Courtillot *et al.*³² pointed out that electrical conductivity must be $< 300 \Omega^{-1} \text{m}^{-1}$ in 97% of the volume of the mantle. A precise determination of mantle filter coefficients will be required before any firmer statement can be made. The complex behaviour of the post-1970 solar magnetic effects (see ref. 27) and the poor geographical distribution of magnetic observatories may render a precise determination difficult.

Variations of westward drift rate

It has been known since the time of Halley (1692) that a significant part of the secular variation field can be accounted for by a westward rotation of the main field about the rotation axis of the Earth, known as westward drift (see ref. 33). Courtillot *et al.*⁶ and Ducruix *et al.*²⁷ proposed that the 1969 impulse could be partly due to an enhancement of westward drift. Following Bullard *et al.*³³ and others, Le Mouél *et al.*³⁴ estimated the westward drift rate w by maximizing the contribution of the term $w \cdot \partial \mathbf{B} / \partial \phi$ (where ϕ is longitude) to secular variation $\dot{\mathbf{B}} = \partial \mathbf{B} / \partial t$. This estimate is:

$$w = \frac{\iint_S \dot{\mathbf{B}} \cdot \partial \mathbf{B} / \partial \phi \, dS}{\iint_S (\partial \mathbf{B} / \partial \phi)^2 \, dS}$$

where the integrals are extended over the entire surface S of the Earth. Estimates can be made either directly from observa-

tory data or spherical harmonic models of the fields $\mathbf{B}$ and $\dot{\mathbf{B}}$. In the last case, one can use the whole field or a single component³⁴. Although results obtained with spherical harmonics were inaccurate and oversmoothed in time, they revealed a consistent pattern of decreasing westward drift from 1955 until 1970–75, increasing since then. Results were clearer with the raw observatory data, where the w variation displayed the characteristic V shape of the original recordings, with a minimum around 1970. The analysis was pushed further by Gire *et al.*³⁵ who used Hodder's³⁶ secular variation coefficients (Fig. 4). Gire *et al.* computed the energy spectrum of the geomagnetic field at the Earth's surface and noted that, regardless of the component being used to estimate w , the major contribution to this estimate came from multipoles with degree <4 . The dominant contribution was found to come from the quadrupolar term P_2^2 , as already discussed, for instance, by Yukutake³⁷, and to a lesser extent from P_2^1 . Gire *et al.* further noted that estimates of w based respectively on Y , B , Z and X were always in the order $w_Y > w_B > w_Z > w_X$. They suggested, based on numerical experiments on existing field and secular variation models, that all these estimates were smaller than the actual w and that Y provided the best estimate. Errors on w_Y estimates in the numerical experiments were of the order of 20%.

The results (Fig. 4) confirm the regular decrease in w from 1950 to 1970, already pointed out by Harwood and Malin²⁵. The decrease started around 1913 as shown by Hodder³⁶, and Gire *et al.*¹⁸. The increase that started in 1970 has now brought the westward drift rate back to its 1950 value.

Motions in fluid core

Secular variation and, in particular, westward drift may provide information on fluid core motions. To extract that information, *a priori* knowledge of the electrical conductivity of the core material is required. This conductivity is estimated to be of the order of 10^5 – $10^6 \Omega^{-1} \text{m}^{-1}$ (see ref. 38), that is $\sim 10^3$ – 10^4 times larger than the estimated conductivity of the lower mantle. On the decade time scale, which is typical of secular variation, the core material is actually often considered to be a perfect conductor. Voorhies and Benton³⁹ have recently shown with MAGSAT data that this is an excellent approximation. In that case, the secular variation observed outside the core depends only on the motion $\mathbf{U}$ at the core–mantle boundary⁴⁰ (CMB), more precisely at the surface of the main stream of the upper core just below a transparent boundary layer⁴¹. Under this so-called frozen-flux approximation^{41,42}, the lines of force of the magnetic field are advected by motions at the surface of the main stream of the core. Many authors propose that the geomagnetic westward drift is due to the drift of the outer core layers with respect to the mantle. It is then tempting to associate secular variation impulses with corresponding impulses in the drift rate of the outer core. Another suggestion put forward by Hide^{43–46} is that westward drift could be due to magnetohydrodynamic waves in the liquid core.

The horizontal velocity field at the CMB can be written as a sum of toroidal terms and of poloidal terms which characterize upwelling, that is exchange of material with the deeper regions of the core. The first toroidal term $t_1^0 \theta_1^0$ characterizes the body rotation part of westward drift. The equation which relates $\mathbf{B}$, $\dot{\mathbf{B}}$ and $\mathbf{U}$ is:

$$\dot{\mathbf{B}}_r = -\text{div}_s(\mathbf{B}_r \mathbf{U}) \quad (1)$$

where div_s is the surface divergence operator ($\nabla \cdot \mathbf{r}(\mathbf{r} \cdot \nabla)$). Since the relative change in $\mathbf{B}$ is comparatively small in the time interval of decades considered here, we might indeed expect that secular impulses in $\dot{\mathbf{B}}$ be related to similar accelerations of $\mathbf{U}$. To continue B_r and $\dot{B}_r$ downward from the surface of the Earth to the CMB, one often assumes that the mantle is a perfect insulator. Although this is clearly not the case, the mantle conductivity is sufficiently weak not to distort the core field significantly. Benton *et al.*^{47,48} and Whaler and Gubbins^{49,50} have continued B_r and $\dot{B}_r$ to the CMB under the assumption of an insulating mantle. Benton and Whaler⁵¹ investigated the effects

of the weakly-conducting mantle using a perturbation procedure and found that, at present, the errors in the core fields due to uncertainties in the higher-order terms of the surface field models are larger than the perturbations due to finite conductivity. Backus (ref. 31 and personal communication) has shown that, provided the delay time τ_l is about the same for individual modes, downward continued maps of B_r and $\dot{B}_r$ at the CMB actually describe the field there as it was τ_l yr before the time when geomagnetic variations occurred at the Earth surface.

Backus⁴² showed that the determination of $\mathbf{U}$ from $\mathbf{B}$ is an ill-posed problem and that if one solution can be found, an infinity of other solutions can be built from it. However, several authors have recently attempted to use the rather simple and well-organized pattern of secular variation to estimate $\mathbf{U}$ using additional restricting assumptions.

Whaler⁵² used equation (1) only at extrema of B_r on the CMB. She noted that if the upper core is stably stratified, that is, if there is no poloidal component in $\mathbf{U}$ ($\text{div}_s \mathbf{U} = 0$), then these extrema must be points at which $\dot{B}_r$ is zero. Using the 1965.0 IGRF model ($N=8$), Whaler noted that ($\dot{B}_r = 0$) contours pass remarkably close to extrema of B_r . She used a statistical argument to suggest that this observation is significant, implying a stably stratified layer at the top of the core. In more recent work, Whaler uses linear inverse theory in an attempt to estimate integrals of B_r inside spherical caps bounded by ($B_r = \text{constant}$) contours (ref. 53 and personal communication). With 318 data points from well-distributed long running permanent observatories, she finds that the error due to imperfect resolution far exceeds that due to mapping of the original data into the solutions and that a considerably larger data set would be needed to decide whether the core velocity field is purely toroidal. This conclusion is independently sustained by work done by Benton *et al.*⁴⁸. These authors show that the shape of null-flux curves ($B_r = 0$ contours) and the number and locations of critical points of B_r are a sensitive function of the truncation level of the spherical harmonic expansion.

Madden and Le Mouél⁵⁴ and Gire *et al.*³⁵ also expanded $\dot{B}_r$ in spherical harmonics at the CMB under the assumption of an insulating mantle and derive (from equation (1)) the linear equations which relate observations $\mathbf{b}$ (the vector of coefficients of the B_r spherical harmonic expansion) to the unknowns $\mathbf{u}$ (the vector of coefficients of the $\mathbf{U}$ expansion in elementary poloidal and toroidal harmonics modes):

$$\mathbf{D} \cdot \mathbf{u} = \mathbf{b} \quad (2)$$

Alternatively, the data can be values of secular variation components at several observatories (140 in Madden and Le Mouél⁵⁴ analysis). Gire *et al.*³⁵ use both a generalized inverse approach^{55,56} and a stochastic inverse approach⁵⁷ to solve equation (2). To reduce the fundamental non-uniqueness in the problem, both Madden and Le Mouél⁵⁴ and Gire *et al.*³⁴ restrict $\mathbf{U}$ to be a simple regular motion by damping the higher degree terms of the truncated expansion.

Gire *et al.*³⁵ concluded that the t_1^0 estimate is stable (stability being defined mathematically) and clearly dominates other terms. The estimate of the drift rate of the outer core layers is of the same order as values computed from observations of geomagnetic westward drift made at the Earth's surface. Estimates for t_1^0 in 1980 are between 0.15 and $0.20^\circ \text{yr}^{-1}$ and are systematically higher than estimates in 1970 (between 0.05 and $0.10^\circ \text{yr}^{-1}$): this acceleration is thought to be linked with the 1969 impulse. This result had not been obtained in the earlier analysis of Madden and Le Mouél⁵⁴. More recently, Gire *et al.* (personal communication) have extended the discussion to higher-order terms, and found that no term of degree higher than four is stable. Gire *et al.* constructed poloidal and toroidal motion models from these stable terms (Fig. 5) and found that between 1970 and 1980 the shape of the toroidal motion (clearly dominated by westward drift) does not change much but that its intensity increases by 70%. In contrast, the geometry of poloidal motions is different. The motion, which had slowed down in 1970, had returned to a stronger, more energetic state

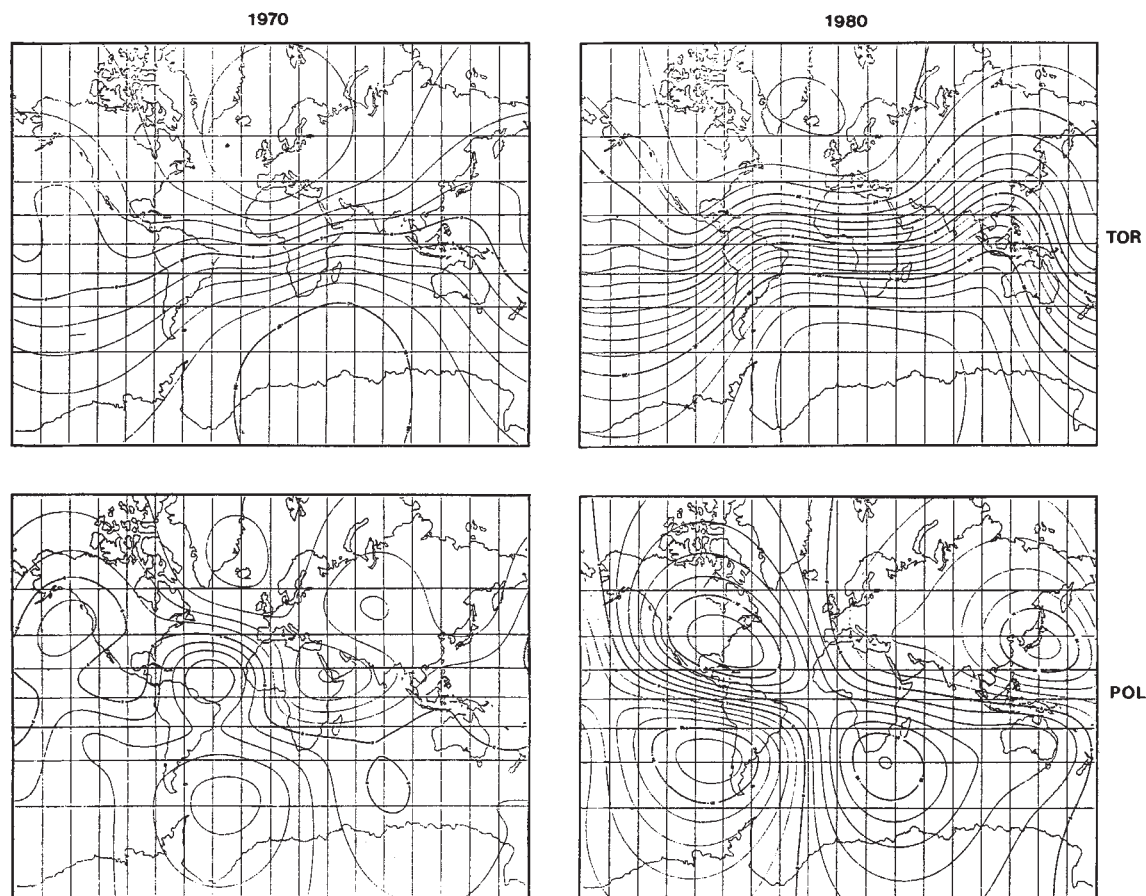


Fig. 6 Stable toroidal and poloidal motion models of Gire *et al.* (personal communication) for 1970 and 1980.

in 1980, the energy input probably being related to the 1969 impulse. Gire *et al.* are able to generate the observed secular variation field from either a purely poloidal or a purely toroidal motion, but this requires harmonics with considerably higher degree and much more energy than the low degree stable composite (toroidal + poloidal) motion. Whaler (personal communication) did not find an acceptable purely toroidal velocity field that would satisfy the surface geomagnetic secular variation data. Velocity fields which satisfied the original data to within their errors had considerable short-wavelength motion that could not possibly be resolved by the data. They were also unstable in the sense that small changes in the misfit between data and model produced very different velocity models.

Thus, parallel studies of recent geomagnetic secular variation suggest that the upper core is not stably stratified and that fluid upwelling exists at the CMB. Even if one is not yet ready to accept the velocity models of Fig. 5 as having actually something to do with real motions, these motions can still be considered as (in a way) the simplest phenomenological representation of the secular variation field.

Rotation of Earth

An up-to-date discussion of the Earth's rotation, in particular of its irregularities, has recently been given by Lambeck⁵⁸ (see also ref. 59). Hide⁴⁴ summarizes aspects of the problem that are relevant to atmosphere-mantle and core-mantle coupling. Small irregular changes in the rotation rate (or corresponding length of day, l.o.d.) can be attributed in part to atmospheric circulation: Hide *et al.*⁶⁰ and Barnes *et al.*⁶¹ have shown that observed short-term changes in l.o.d. (with a period less than a few months and perhaps up to 1 yr) can be fully accounted for on the basis of angular momentum exchange of the mantle and atmosphere. On the other hand, the observed changes in l.o.d. of up to 5 ms on the decade time scale⁶² cannot be associated with atmosphere-mantle coupling⁵⁸. There is a general consensus that these

variations originate in the Earth's core in relation to geomagnetic field generation and electromagnetic or mechanical core-mantle coupling. Hide⁴⁴ reviews the magnitudes of three kinds of horizontal stresses that couple the core to the mantle finding that viscous coupling is almost certainly inadequate. Electromagnetic coupling is sufficient (provided available estimates of relative electrical conductivities and field intensities are correct), although topographic coupling due to bumps on the core-mantle interface of about 1 km in height may be important^{45,46}. Therefore, we may expect to find a correlation between geomagnetic indicators and changes in l.o.d. in the decade period range which is typical of secular variation. Vestine⁷ was probably the first to give evidence that such a correlation might exist.

The discovery of the 1969 geomagnetic impulse has revived this topic. Courtillot *et al.*⁶ noted a striking proximity between periods of intense secular acceleration changes, particularly impulses, and extrema in the l.o.d. variations. Le Mouél *et al.*⁶³ compared the secular variation of D in Europe and l.o.d. (with a sign error in their figure, see ref. 64) over the past 120 yr, and found a good correlation, with l.o.d. variations lagging geomagnetic variations by 10–15 yrs (Fig. 6). Le Mouél *et al.*⁶³ have suggested that secular variation impulses near 1900 (?), 1913, and 1969 should be linked with extrema of the l.o.d. curve near 1910, 1930 and 1980 (?): increased (decreased) westward drift is followed by increased (decreased) rotational velocity of the Earth. This correlation is the opposite of that proposed by Vestine⁷, Runcorn (ref. 65 and personal communication) and more recently by Backus³¹. Backus has pointed out that Morrison's⁶² l.o.d. data indicate that the angular position of the mantle suffered an impulse of order 3 in 1956 and suggested that both this event and the 1969 geomagnetic impulse may be caused by an impulse in westward drift: in that case, the magnetic effects would lag the rotational effects by some 13 yr, an increase in westward drift following a decrease in rotational velocity.

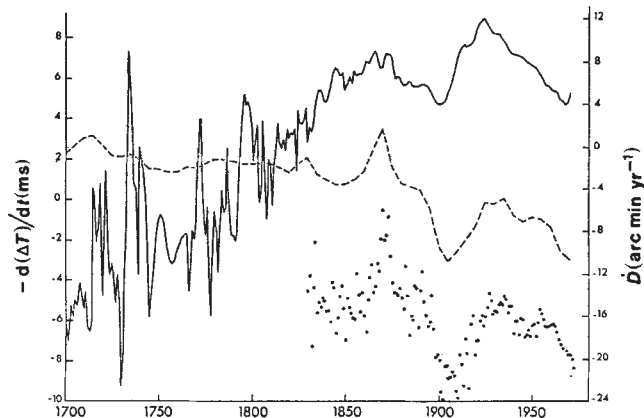


Fig. 6 Plot of the secular variation of declination $\dot{D}$ at Chambon La Forêt (solid curve) and of the excess length of day $-d(\Delta T)/dt$ (ref 62) (dashed curve) for the period 1700–1972 (see refs 63, 64 for the sign correction which is included here). Dots are annual means of excess l.o.d. from which the smooth dashed curve is derived⁶². For clarity they are displaced by 5 ms.

One way to assess the correlation is to investigate whether it extends back in the past. As already pointed out, the number of available observatories before 1900 is limited, but a preliminary analysis of the 1700–1870 period (ref. 63 and J.L. Le M. *et al.*, in preparation) seems to indicate that this was a time with no dramatic geomagnetic changes, no large accelerations of westward drift, and also no large changes in the acceleration of the Earth's rotation (Fig. 6). It may then be tentatively suggested that the correlation still holds qualitatively and that the eighteenth and nineteenth centuries were times when the core regime was more stable than in the present century.

Models of core–mantle coupling

Simple models of mechanical and electromagnetic core–mantle coupling can be constructed in an attempt to account roughly for the magnitude of l.o.d. and westward drift variations and for the correlation which is assumed to link the two. If one accepts the correlation which is suggested by Backus and Vestine, the fact that changes in westward drift rate follow l.o.d. changes and are of the same sign can be understood if one assumes convective mass exchange between a thin rigid shell, forming the top of the core, and the rest of the liquid core below it (called here a three-layer model). This model is similar to that originally proposed by Bullard⁶⁶ and later extended by Rochester⁶⁷. The radial mass exchange exerts a torque on the outer core and (in the case of 1956 l.o.d. event studied by Backus) decelerates it. Electromagnetic coupling at the CMB in turn decelerates the mantle. The changes in rotational velocity of the mantle would immediately show as l.o.d. changes (a second-order impulse in mantle angular velocity in 1956), and be followed by geomagnetic changes, observed with a 13-yr mean delay due to diffusion of westward drift related signals through a rather strongly conducting mantle³¹.

If, on the other hand, one follows the correlation suggested by Le Mouél *et al.*⁶³, one has to account for the discrepancy between the short time scale over which the geomagnetic impulse is established (1 yr) and the long delay after which the rotation of the mantle is altered (10–15 yr). We have also used a three-layer model⁶⁸, in which an impulsive torque (P) related to core convection is applied to the top core layer and a torque ($-P$) to the rest of the liquid core. When the torques exerted by Lorentz forces on the three part of this electromagnetic system are computed, a linear differential system for the rotations of the mantle and of the top and rest of the liquid core results. We⁶⁸ found that the solutions of this system depend exponentially on two time constants, one always short (<1 yr) and the other proportional to the ratio σ_c/σ_m of the core versus mantle conductivity. If this time constant can be identified with the 10–15 yr delay in changes of l.o.d., σ_c/σ_m is found to range

from 500 to 1,000, in agreement with conductivity estimates discussed above. For (P) a first-order impulse (that is a step function), the l.o.d. suffers a second-order impulse, and the ratio $(\delta\omega_m)/(\delta\omega_0)$ of asymptotic amplitudes of rotational changes in the mantle and outer core layer following the impulse is found to depend only, in a first-order approximation, on the ratio of the outer core layer thickness h to the total core radius r_c . We found h to be of the order of 150 km. The radial velocity of fluid exchange between the outer and inner parts of the external core was found to be $\sim 10^{-6}$ ms⁻¹. Although this model provides a reasonable account for observations discussed by Le Mouél *et al.*⁶³, it is limited in that it does not include a quantitative treatment of magnetic field transport, particularly from the outer–inner core interface to the CMB. Thus, both an improved assessment of the l.o.d./geomagnetic correlation and more complete models of electromagnetic core–mantle coupling are required.

Runcorn (ref. 65 and personal communication) argues that irregular changes in l.o.d. may even be shorter than the present smoothing of the astronomical data indicates. If such impulsive torques indeed act on the mantle to provide changes in l.o.d., Runcorn points out that equatorial components of these torques may exist and excite the Chandler wobble. He proposes that such an impulse occurring in mid-1967 in the Chandler wobble supports this idea. It is not clear whether this event can be causally related to the 1969 geomagnetic impulse. On the other hand, Barnes *et al.*⁶¹ showed that meteorological phenomena provide an important contribution to the excitation of polar motion.

Both Runcorn (personal communication) and Backus (ref. 69 and personal communication) note that the magnetic variations related to the 1969 impulse should be accompanied by a second-order impulse in the tangential component of the electric field on the ocean bottom, with an amplitude of the order of 10^{-8} Vm⁻¹ yr⁻¹. Runcorn and colleagues (personal communication) have observed the occurrence in 1969 of step-like increases of d.c. potentials on long telegraph cables in the southwestern Pacific. These might arise from leakage currents associated with the core magnetic field and might be related to the geomagnetic impulse (Runcorn *et al.* in preparation).

Conclusion and perspectives

The reality of short-term (quasi-discontinuous) changes in the rate of internal geomagnetic secular variation has been confirmed by observational evidence in the past few years. The last worldwide impulse occurred in 1969 and may have been preceded by an event in 1913. The duration (of the order of 1 yr), intensity and geographical pattern of these impulses constrain values of deep mantle conductivity and the pattern of flow in the upper part of the fluid core. We suggest a correlation of geomagnetic westward drift and the l.o.d.; this correlation is still debated but would place important constraints on mechanisms of core–mantle coupling. It has also been proposed⁷⁰ that decade fluctuations of climate and l.o.d. are both correlated with geomagnetic changes, thus raising the possibility of a long-term influence of core motions on some climatic trends. In this process, angular momentum would be transferred from the core to the mantle and to the atmosphere.

Theoretical work³¹ indicates the need for further observational work on both recognized impulses and earlier (seventeenth to nineteenth century) events, although the data base is scarce. Present data favour the existence of upwelling in the fluid upper core. Secular variation models which involve impulsive events separated by quiet phases are simpler, both in space and time, than previously expected and yet account for much of the energy of observed secular variation.

Progress on dynamo theory in coming years might be expected to come from detailed observational advances of two basic features of the geomagnetic field: impulses and reversals. The frozen-flux approximation, which is valid for impulses, might be extended to the 1,000-yr time scale which characterizes reversals. Much progress has been made on the description of

individual reversals (see refs 71–73) and on the frequency of reversals (see refs 74, 75). The interests and frequency bands of specialists of geomagnetism and palaeomagnetism are converging in this endeavour.

This paper reviews published work on geomagnetic impulses and contributions to a meeting held on 27 September 1983 at the Institut de Physique du Globe in Paris on the occasion of the centenary of the French National Magnetic Observatory. Our review has greatly benefited from oral presentations made at the meeting, and from written communications sent after the meeting by G. Backus, R. Hide, W. Lowrie, T. Madden, S. Malin, K. Runcorn and K. Whaler. The meeting and paper are dedicated to the observers in magnetic observatories whose

painstaking and often ill-recognized efforts in gathering data are essential for the kind of work described here. IGP contribution NS793.

Note added in proof: M. McLeod (personal communication) has completed a detailed response to Alldredge's criticism¹⁷ which fully vindicates our analyses^{6,13} and those of Malin *et al.*^{14,17}. The following conclusions from McLeod's work are worth noting: (1) The 1969 impulse is clearly real and of internal origin. The external coefficients of the s.h. expansion¹⁷ appear to represent noise due to external currents unrelated to the impulse. Better estimates of internal s.h.c. are obtained. (2) The 1969 impulse can be as short as 1 yr in duration and its date of occurrence is found to be 1969.5.

1. Currie, R. G. *J. geophys. Res.* **71**, 4579–4598 (1966).
2. Currie, R. G. *Astrophys. Space Sci.* **21**, 425–438 (1973).
3. Alldredge, L. R. *J. Geomagn. Geoelectr.* **29**, 123–135 (1977).
4. Yukutake, T. *J. Geomagn. Geoelectr.* **17**, 287–309 (1965).
5. Courtillot, V. & Le Mouél, J. L. *J. geophys. Res.* **81**, 2941–2950 (1976).
6. Courtillot, V., Ducruix, J. & Le Mouél, J. L. *C. r. hebdom. Séanc. Acad. Sci., Paris D287*, 1095–1098 (1978).
7. Vestine, E. H. *Proc. natn. Acad. Sci. U.S.A.* **38**, 1030–1038 (1952).
8. Walker, J. B. & O'Dea, P. L. *EOS* **33**, 797–800 (1952).
9. Runcorn, S. K. *EOS* **36**, 191–198 (1955).
10. McDonald, K. L. *J. geophys. Res.* **62**, 117–141 (1957).
11. Alldredge, L. R. *J. geophys. Res.* **80**, 1571–1578 (1975).
12. Alldredge, L. R. *C. r. hebdom. Séanc. Acad. Sci., Paris B289*, 169–172 (1979); *J. geophys. Res.* **89**, 4403–4412 (1984).
13. Courtillot, V., Ducruix, J. & Le Mouél, J. L. *C. r. hebdom. Séanc. Acad. Sci., Paris B289*, 173–175 (1979).
14. Malin, S. R. C., Hodder, B. M. & Barraclough, D. R. in *75th Anniversary Volume of Ebro Observatory*, (ed. Cardus, J. O.) (Tarragona, in the press).
15. Duyen Chau, Ducruix, J. & Le Mouél, J. L. *C. r. hebdom. Séanc. Acad. Sci., Paris B293*, 157–160 (1981).
16. Le Mouél, J. L., Ducruix, J. & Duyen, C. *Phys. Earth planet. Inter.* **28**, 337–350 (1982).
17. Malin, S. R. C. & Hodder, B. M. *Nature* **296**, 726–728 (1982).
18. Gire, C., Le Mouél, J. L. & Ducruix, J. *Nature* **307**, 349–352 (1983).
19. Ducruix, J., Gire, C. & Le Mouél, J. L. *C. r. hebdom. Séanc. Acad. Sci., Paris B296*, 1419–1424 (1983).
20. Malin, S. R. C. & Bullard, E. C. *Phil. Trans. R. Soc. A299*, 357–423 (1981).
21. Hodder, B. *Geophys. J. R. astr. Soc.* **65**, 763–776 (1981).
22. Lahiri, B. N. & Price, A. T. *Phil. Trans. R. Soc.* **237**, 509–540 (1939).
23. Alldredge, L. R. *J. geophys. Res.* **81**, 2990–2996 (1976).
24. Alldredge, L. R. *J. geophys. Res.* **82**, 5427–5431 (1977).
25. Harwood, J. M. & Malin, S. R. C. *Nature* **259**, 469–471 (1976).
26. Courtillot, V. & Le Mouél, J. L. *J. geophys. Res.* **84**, 4785–4790 (1979).
27. Ducruix, J., Courtillot, V. & Le Mouél, J. L. *Geophys. J. R. astr. Soc.* **61**, 73–94 (1980).
28. Achache, J., Le Mouél, J. L. & Courtillot, V. *Geophys. J. R. astr. Soc.* **65**, 579–601 (1981).
29. Courtillot, V., Ducruix, J., Le Mouél, J. L. & Achache, J. in *Mechanisms of Continental Drift and Plate Tectonics*, (eds Davies, P. A. & Runcorn, S. K.) 317–326, (Academic, New York, 1980).
30. Achache, J., Courtillot, V., Ducruix, J. & Le Mouél, J. L. *Phys. Earth planet. Inter.* **23**, 72–75 (1980).
31. Backus, G. *Geophys. J. R. astr. Soc.* **74**, 713–746 (1983).
32. Courtillot, V., Le Mouél, J. L. & Ducruix, J. *Geophys. J. R. astr. Soc.* **78**, 619–625 (1984).
33. Bullard, E. C., Freedman, C. F., Gellman, H. & Nixon, J. *Phil. Trans. R. Soc. A243*, 67–92 (1950).
34. Le Mouél, J. L., Ducruix, J. & Duyen, C. *Geophys. Res. Lett.* **10**, 369–372 (1983).
35. Gire, C., Le Mouél, J. L. & Madden, T. *Annls Geophys.* **2**, 37–46 (1984).
36. Hodder, B. *Nature* **306**, 136–137 (1983).
37. Yukutake, T. *J. Geomagn. Geoelectr.* **25**, 195–212; 231–235 (1973).
38. Jeanloz, R. *Scient. Am.* **249**, 40–49 (1983).
39. Voorhies, C. V. & Benton, E. R. *Geophys. Res. Lett.* **9**, 258–261 (1982).
40. Bondi, H. & Gold, T. *Mon. Not. R. astr. Soc.* **110**, 607–611 (1950).
41. Roberts, P. H. & Scott, S. J. *Geomagn. Geoelectr.* **17**, 137–151 (1965).
42. Backus, G. *Phil. Trans. R. Soc. A263*, 239–266 (1968).
43. Hide, R. & Stewartson, K. *Rev. Geophys. Space Phys.* **10**, 579–598 (1972).
44. Hide, R. *Phil. Trans. R. Soc. A299*, 233–234 (1982); *IUGG Chronicle*, (ed. Melchior, P.) (in the press).
45. Malin, S. R. C. & Hide, R. *Phil. Trans. R. Soc. A299*, 281–287 (1982).
46. Hide, R. *Q. Jl. R. Soc.* **96**, 579 (1970).
47. Benton, R. E., Muth, L. A. & Stix, M. J. *Geomagn. Geoelectr.* **32**, 615–626 (1979).
48. Benton, R. E., Estes, R. H., Langel, R. A. & Muth, L. A. *Geophys. Res. Lett.* **9**, 254–257 (1982).
49. Whaler, K. A. *Phil. Trans. R. Soc. A306*, 235–246 (1982).
50. Gubbins, D. *Phil. Trans. R. Soc. A306*, 247–254 (1982); *Geophys. J. R. astr. Soc.* **77**, 753–766 (1984).
51. Benton, R. E. & Whaler, K. A. *Geophys. J. R. astr. Soc.* **75**, 77–100 (1983).
52. Whaler, K. A. *Nature* **287**, 528–530 (1980).
53. Whaler, K. A. *Geophys. J. R. astr. Soc.* **78**, 453–474 (1984).
54. Madden, T. & Le Mouél, J. L. *Phil. Trans. R. Soc. A306*, 271–280 (1982).
55. Backus, G. & Gilbert, F. *Geophys. J. R. astr. Soc.* **16**, 169–205 (1968); *Phil. Trans. R. Soc. A266*, 123–192 (1970); *Geophys. J. R. astr. Soc.* **13**, 247–276 (1967).
56. Jackson, D. D. *Geophys. J. R. astr. Soc.* **28**, 97–110 (1972).
57. Jackson, D. D. *Geophys. J. R. astr. Soc.* **57**, 137–157 (1979).
58. Lambeck, K. *The Earth's Variable Rotation: Geophysical Causes and Consequences* (Cambridge University Press, New York, 1980).
59. Cazenave, A. thesis, Univ. Toulouse (1975).
60. Hide, R., Birch, N. T., Morrison, L. V., Shea, D. & White, A. A. *Nature* **286**, 114–117 (1980).
61. Barnes, R. T. H., Hide, R., White, A. A. & Wilson, C. A. *Proc. R. Soc. A387*, 31–73 (1983).
62. Morrison, L. V. *Geophys. J. R. astr. Soc.* **58**, 349–360 (1979).
63. Le Mouél, J. L., Madden, T. R., Ducruix, J. & Courtillot, V. *Nature* **290**, 763–765 (1981).
64. Courtillot, V., Le Mouél, J. L., Ducruix, J. & Cazenave, A. *Nature* **303**, 638 (1983).
65. Runcorn, S. K. *Phil. Trans. R. Soc.* **306**, 261–270 (1982).
66. Bullard, E. C. *Mon. Not. R. astr. Soc., Geophys. Suppl.* **5**, 248–257 (1948).
67. Rochester, M. G. *Phil. Trans. R. Soc.* **252**, 531–555 (1960); *J. geophys. Res.* **17**, 4833–4836 (1962).
68. Le Mouél, J. L. & Courtillot, V. *Phys. Earth planet. Inter.* **24**, 236–241 (1981); *J. geophys. Res.* **87**, 4103–4108 (1982).
69. Backus, G. *Phys. Earth planet. Inter.* **28**, 191–214 (1982).
70. Courtillot, V., Le Mouél, J. L., Ducruix, J. & Cazenave, A. *Nature* **297**, 386–387 (1982).
71. Hoffman, K. *Rev. Geophys. Space Phys.* **21**, 614–620 (1983).
72. Valet, J. P., Laj, C. & Langeris, C. G. *Nature* **304**, 330–332 (1983).
73. Prévôt, M., Mankinen, E. A., Grommé, C. S. & Coe, R. S. *EOS* **63**, 921 (1982).
74. Lowrie, W. & Kent, D. V. *Earth planet. Sci. Lett.* **62**, 305–313 (1983).
75. Mazaud, A., Laj, C., de Sèze, L. & Verosub, K. L. *Nature* **304**, 328–330 (1983).

ARTICLES

Cell type-specific activation of actin genes in the early amphibian embryo

T. J. Mohun, S. Brennan, N. Dathan, S. Fairman & J. B. Gurdon

CRC Molecular Embryology Research Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

Muscle actin genes are the earliest yet described to show cell type-specific activation in amphibian embryos. Gene-specific probes show that α -skeletal and α -cardiac actin genes start to be transcribed simultaneously at the end of gastrulation, but only in those regions of the mesoderm that subsequently form embryonic muscle. Their expression provides a molecular marker for early cell determination.

WITHIN a day of egg fertilization, most animal embryos comprise many morphologically distinct tissues and organ primordia, the arrangement of which remains essentially unchanged throughout all subsequent development¹. In each region of the embryo, cells initiate distinct programmes of gene expression as they embark on diverse pathways of differentiation, the basic

body plan of the embryo being laid down from the earliest times. As yet, however, almost nothing is known of the molecular mechanisms that underlie regional differences in cell fate within the early embryo. Classical theories suggest that the spatial pattern of early cell differentiation reflects the distribution of cytoplasmic factors or 'determinants' inherited by the early

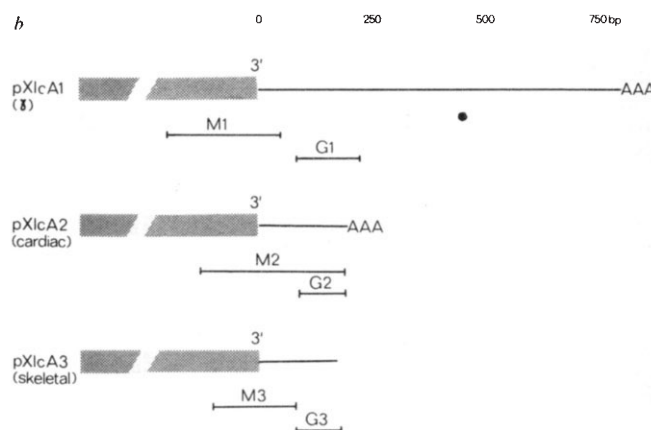
a	amino acid number	1	5	10
cytoskeletal actin (γ)*		Ala Glu Glu Glu Ile Ala	ALA LEU VAL	Ile ASP.....
cardiac actin*		Asp Asp Glu Glu Thr Thr	ALA LEU VAL	Cys ASP.....
pXlcA1 (Cytoskeletal)		Ala Glu Glu Glu Ile Ala	GCG CTC GTC	Ile GAC.....
		GCA GAA GAA GAG ATT GCA		Cys GAC.....
pXlcA2 (Cardiac)		Asp Asp Glu Glu Thr Thr	GCC TTG GTG	TGC GAC.....
		GAC GAC GAG GAG ACT ACC		

amino acid number	265	270	294	356
cytoskeletal actin (γ)*	PHE	Leu GLY MET GLU SER	Cys GLY....ALA ASN	Thr VALILE Ser LYS GLN.....
cardiac actin*	PHE	Ile GLY MET GLU SER	Ala GLY....ALA ASN	Asn VALILE Ser LYS GLN.....
pXlcA1 (Cytoskeletal)	TTC	Leu GGT ATG GAA TCC	Cys GGC....GCC AAC	Thr GTCATC Ser AAG CAA
		CTG Ile	Ala	Asn
pXlcA2 (Cardiac)	TTC	ATT GGT ATG GAA TCT	GCC GGT....GCC AAT	AAT GTCATC Ser AAG CAA
		Ile	Ala	Asn
pXlcA3 (Skeletal)	TTC	ATT GGC ATG GAA TCT	GCT GGC....GCC AAC	AAC GTTATC Ser AAG CAA
				ACC

* consensus actin sequence for vertebrates

boxes indicate diagnostic amino acids

Fig. 1 Identification of *Xenopus* actin cDNAs. **a**, Portions of the nucleotide sequence for three *Xenopus* actin cDNAs, pXlcA1, pXlcA2 and pXlcA3, obtained using the dideoxy sequencing procedure^{32,33}. These are aligned by comparing their coding capacity with the consensus polypeptide sequence of vertebrate γ -cytoskeletal and α -cardiac actins⁹⁻¹³. Amino acids diagnostic for particular actin protein types are boxed. Diagrams of each cDNA are shown in **b**. Amino acid coding regions are indicated by stippling. Subclones used in subsequent experiments are identified; M1, M2 and M3 contain the 3' terminal portions of protein coding sequence and an adjacent region of the untranslated mRNA trailer. These were used for S₁ nuclease protection experiments. G1, G2 and G3 comprise subclones for the mRNA 3'-untranslated region and provided gene-specific hybridization probes in RNA blot experiments.



blastomeres², but few of these have been demonstrated in any organism and none has been purified. The nature of determinants and the manner by which they regulate cell differentiation remain central problems for embryologists.

As a prerequisite for a molecular analysis of embryonic cell differentiation we have examined the expression of the actin multi-gene family in the amphibian embryo. It is well established that among vertebrates the several actin proteins are distributed in a tissue-specific manner^{3,4}. The cytoskeletal actins are ubiquitous, whereas the sarcomeric actins are found only in muscle cells where they constitute a major component of the contractile apparatus. In amphibians, the morphological differentiation of cells that give rise to embryonic muscle occurs soon after neurulation^{5,6} and the onset of muscle actin protein synthesis⁷. We report here that activation of amphibian α -cardiac and α -skeletal actin genes is coordinated at the end of gastrulation. Using mRNA-specific probes derived from *Xenopus laevis* α -cardiac, α -skeletal and γ -cytoskeletal actin cDNAs, we find that activation of both muscle-specific genes is precisely localized to certain regions within the mesoderm. In contrast, cytoskeletal actin gene transcription can be detected throughout the entire embryo.

Characterization of actin cDNAs

cDNA libraries from embryo RNA and adult skeletal muscle RNA were constructed as described elsewhere (T.J.M. *et al.*, in preparation) and screened for actin cDNA sequences using a chick β -actin cDNA⁸ as probe. The entire DNA sequence of several positive recombinants was determined to establish the

amino acid sequence of the actin that each cDNA encoded. Amongst vertebrates, the two cytoskeletal (designated β and γ) and four sarcomeric actins (α -cardiac, α -skeletal and two smooth muscle types) may be identified by characteristic differences in their polypeptide sequence⁹⁻¹³. Using these diagnostic differences, we obtained a tentative identification of three *Xenopus* cDNAs, pXlcA1-3. pXlcA1 codes for a protein identical to the consensus for vertebrate γ -cytoskeletal actins (Fig. 1a). Unlike other vertebrates, amphibians possess a variety of cytoskeletal actins that differ from both β -type and γ -type proteins¹⁴. pXlcA2 and pXlcA3 match the consensus for muscle-type actins, pXlcA2 coding for α -cardiac actin and pXlcA3 encoding the related α -skeletal actin.

In addition to determining the coding capacity of each cDNA, we have examined the untranslated portions of their sequence (T.J.M. *et al.*, in preparation). We find no homology between any of the three cDNAs in their 3'-untranslated regions; therefore we constructed mRNA-specific hybridization probes for subsequent experiments (Fig. 1b).

Tissue-specific expression of actin mRNAs

We next sought the *in vivo* identity of the three actin cDNAs by determining in which adult tissues their equivalent mRNAs could be detected. RNA from a variety of adult frog tissues was hybridized with individual actin mRNA-specific single-stranded DNA probes M1, M2 and M3, derived from the cDNAs pXlcA1-3. Each probe, comprising several hundred nucleotides, includes the carboxy-terminus of the actin protein as well as a portion

of the adjacent message-specific 3'-untranslated region subcloned into m13mp8. Individual actin mRNAs were identified by the size of the S_1 nuclease-resistant fragments obtained (Fig. 2). As expected, the two muscle-type actin transcripts show distinct, tissue-specific patterns of expression consistent with the amino acid sequences they encode. mRNA corresponding to pXlcA2 is found exclusively in heart tissue (Fig. 2a), whilst the message-specific probe from pXlcA3 identifies transcripts only in skeletal muscle (Fig. 2b). We conclude that the clones

pXlcA2 and pXlcA3 comprise α -cardiac and α -skeletal actin cDNAs respectively. The S_1 nuclease assay shows that α -cardiac and α -skeletal actin mRNAs are at least 50 times less abundant (if present at all) in non-muscle tissues. An equivalent survey demonstrates the presence of cytoskeletal actin transcripts complementary to pXlcA1 in all adult tissues examined (data not shown).

Actin gene expression in embryos

In adult frog tissues, sarcomeric and cytoskeletal actin genes show distinct, tissue-specific patterns of regulation. To discover when these differences are first established, we have examined the levels of cytoskeletal, α -cardiac and α -skeletal actin mRNA in RNA extracted from early embryos using RNA blots and S_1 nuclease protection. We detect actin transcripts of three distinct sizes in neurula embryos using a chick β -actin cDNA probe (Fig. 3). To identify each size class and to determine their presence during the course of early development, we have constructed message-specific probes comprising 100–150 nucleotides of the 3'-untranslated sequence from each of the *Xenopus* actin cDNAs (subclones G1, G2 and G3; see Fig. 1). The specificity of each probe was confirmed by genomic Southern blots; each hybridized to a different subset of restriction fragments (data not shown). The three probes were then hybridized individually to nitrocellulose blots of embryonic RNA derived from several stages of early development (Fig. 3).

Our results confirm that *Xenopus* cytoskeletal and α -cardiac actin mRNAs are similar in size to their counterparts in other vertebrates. The cytoskeletal actin transcript of embryos comprises ~2,300 nucleotides whilst the α -cardiac transcript is ~1,600 nucleotides long and co-migrates with its α -skeletal counterpart (data not shown). An intermediate-sized transcript is also detected throughout early development and presumably encodes a further class of cytoskeletal actins. Even in early embryos, the cytoskeletal and muscle-specific actin genes show different patterns of expression. The α -cardiac actin transcripts and their α -skeletal counterparts are absent in the early stages of development, being first detected in neurula embryos. By contrast, a low, constant level of cytoskeletal actin mRNA was detected in all stages before gastrulation. In later stages, the levels of both cytoskeletal and sarcomeric actin mRNAs increase in a similarly dramatic manner.

The time of actin gene activation

Are the actin genes activated specifically during early embryogenesis or do they simply respond to a 'signal' that stimulates embryonic gene transcription in general? We have made careful estimates of the levels of all three actin mRNAs through development and compared their developmental profiles with those of other identified genes. By a quantitative S_1 nuclease protection assay using single-stranded probes radioactively labelled through their entire length, we have obtained a sensitivity 10-fold greater than that provided by RNA blots. We compared the signals derived from each RNA sample with those of a dilution series using tailbud RNA (stage 26), performing each measurement on RNA extracted from individual, staged embryos (see Fig. 4).

Both α -cardiac and α -skeletal actin mRNAs are first detected in stage 14 embryos which have just completed gastrulation, and the two mRNAs increase their subsequent levels in parallel. The observed actin mRNA development profiles diverge from the theoretical curve for a stable gene transcript at this stage, either because of specific activation of the actin genes at the end of gastrulation, or from a similarly timed and equally dramatic change in the kinetics of actin message turnover. From the specific activity of our probes, we believe that a stage 18 (neurula) embryo contains 10^7 – 10^8 α -cardiac transcripts; this number would be accumulated if the α -cardiac actin gene is single copy and activated in ~5% of cells at stage 13 (activated exclusively in the presumptive somatic mesoderm 1 h before the first transcripts are actually detected). For such a calculation, we assume that stable transcripts are synthesized at a rate of 10

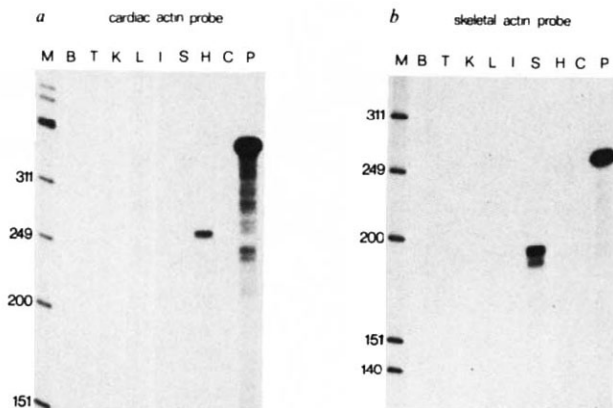


Fig. 2 Tissue-specific expression of actin genes. *a*, 250-nucleotide fragment obtained by protection of the α -cardiac probe M2 by α -cardiac mRNA; *b*, doublet of 185 and 190 nucleotides obtained by protection of the α -skeletal actin cDNA probe M3 by α -skeletal actin mRNA. RNA was prepared from various adult frog tissues and hybridized separately to the two probes M2 and M3 (see Fig. 1). DNA protected from subsequent digestion by S_1 nuclease was analysed by electrophoresis. The source of each RNA sample is indicated. B, brain; T, testes; K, kidney; L, liver; I, intestine; S, leg muscle; H, heart. Lanes 'P' and 'C' are controls using yeast tRNA for the hybridization, before and after S_1 digestion respectively. Size markers were provided by Φ X174 RF DNA digested with *Hinf*I (lane M). Each of the probes M2 and M3 contains 55 nucleotides of M13 sequence which is removed from all DNA/RNA hybrids by S_1 nuclease action. Protected fragments resulting from hybridization of the probes to their corresponding mRNAs are therefore significantly shorter than the input probes. The α -cardiac cDNA pXlcA2 contains an internal polyadenylation signal sequence 91 nucleotides upstream from the poly(A) tail (T.J.M. *et al.*, in preparation); our unpublished experiments indicate that this first polyadenylation signal predominates in transcript processing. Polyadenylation mostly occurs about 15 nucleotides downstream from this first pentanucleotide AAUAA and about 70 nucleotides upstream of the second polyadenylation site. Hence protection of the α -cardiac probe M2 (381 nucleotides) by α -cardiac mRNA yields a 250-nucleotide fragment (*a*). In addition, several fragments approximately 120–130 nucleotides long are protected by α -cardiac actin mRNA (not included in *a*, see Fig. 6). Experiments using end-labelled probes have demonstrated that these result from internal S_1 nuclease cleavage over a region of about five nucleotides in the middle of the actin cDNA portion of probe M2. Protection of the α -skeletal actin cDNA probe M3 (247 nucleotides) by α -skeletal actin mRNA results in a doublet of 185 and 190 nucleotides (*b*), presumably due to minor nibbling at the ends of the 190-base pair DNA/RNA hybrid by S_1 nuclease. RNA was extracted from various adult tissues by homogenization of frozen tissue in 3 M lithium chloride, 6 M urea, 0.5% SDS, 70 mM 2-mercaptoethanol, 10 mM sodium acetate, pH 5.0 (ref. 34). After precipitation overnight at 4 °C, the RNA was resuspended in 0.2% SDS, 100 mM sodium acetate, pH 5.0, extracted twice with phenol and chloroform and reprecipitated. Single-stranded hybridization probes were prepared using subclones M2 and M3 (see Fig. 1) inserted into the *Sma*I site of M13mp8 (ref. 35). Radioactive probes were excised by digestion with *Eco*RI. For S_1 nuclease protection analysis³⁶, 2.5 μ g of total tissue RNA were hybridized with 100,000 c.p.m. of probe in 20 μ l buffer (50% formamide, 1 mM EDTA, 0.4 M NaCl, 10 mM PIPES, pH 7.0) at 30 °C (25 °C below T_m for a DNA:DNA hybrid) overnight. Unhybridized material was digested with 100 units of S_1 nuclease (BRL) at 37 °C for 30 min. S_1 -resistant DNA was collected by ethanol precipitation and analysed by electrophoresis on 6% acrylamide, 8.3 M urea gels.

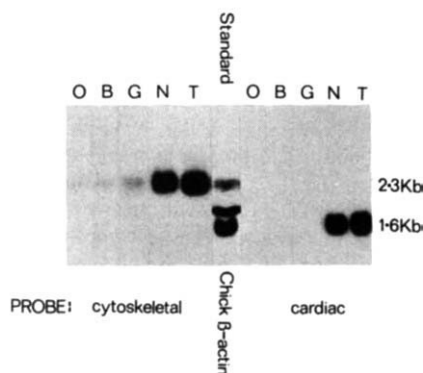


Fig. 3 Actin mRNA accumulation in early embryos. Total RNA was extracted from embryos at successive stages of development as previously described³⁴, resolved by electrophoresis using formaldehyde-agarose gels³⁷ and transferred to nitrocellulose³⁸. The RNA blots were hybridized individually with mRNA-specific probes (G1 and G2) from the α -cardiac and cytoskeletal actin cDNAs (see Fig. 1 legend). Single-stranded, ³²P-labelled probes were synthesized³⁵ in M13mp8. A further blot containing RNA extracted from a neurula embryo was hybridized with the chick β -actin cDNA probe to serve as a standard that identifies all actin mRNA size classes. The figure is a composite from these experiments, using 10 μ g of RNA from fully grown oocytes (O), blastula (B), gastrula (G), neurula (N) and tailbud (T) embryos. Sizes of hybridizing transcripts were estimated by comparison with ribosomal and transfer RNA markers.

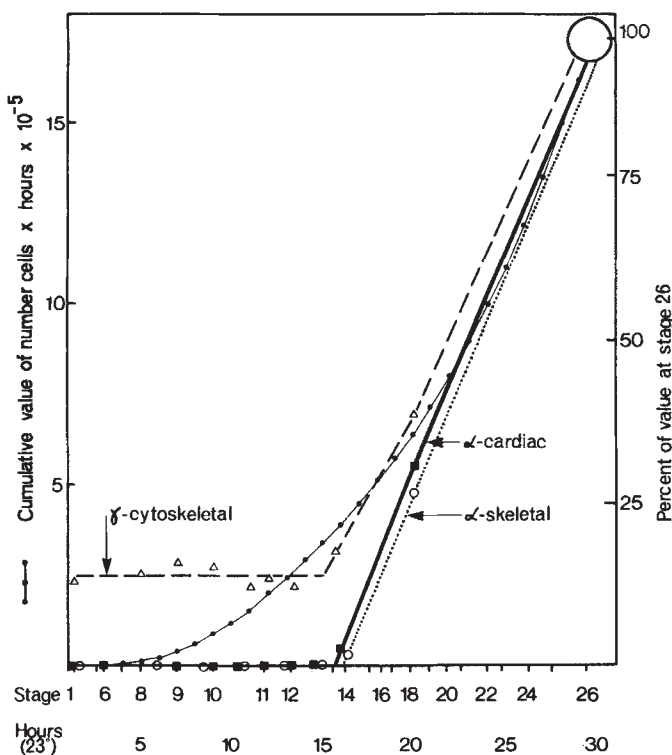


Fig. 4 Temporal changes in actin mRNA content during *Xenopus* development. Relative levels of accumulated actin message were assayed by S_1 nuclease protection as described in Fig. 2 legend, each experiment using the RNA from a single embryo. We confirmed that hybridization signals increased in a linear fashion with input RNA by including a dilution series of RNA from several embryonic stages. Each point represents the average of several determinations in different experiments. These are plotted as a percentage of the value at stage 26. Δ — Δ , Cytoskeletal actin mRNA; $\blacksquare$ — $\blacksquare$, α -cardiac actin mRNA; $\circ$ — $\circ$, α -skeletal actin mRNA. A theoretical accumulation curve ($\bullet$ — $\bullet$) (prepared by adding cumulatively the number of nuclei present at each hour of development) has been superimposed for comparison.

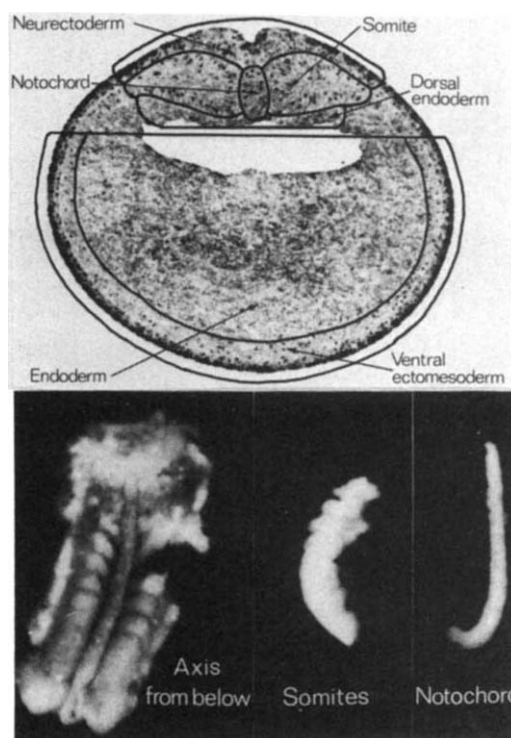


Fig. 5 Dissection of a stage 18 neurula embryo. A transverse section of a stage 18 neurula embryo (1.2 mm diameter) indicating the six fragments that were obtained by dissection, several of which are in the lower figure. After removal of endoderm, ventral ectomesoderm and dorsal endoderm, the remaining fragment (axis) is readily separated into somites, notochord and neurectoderm.

per gene per min¹⁵. Other genes active in early embryos commence transcription at varying times before muscle actin transcripts are first detected, irrespective of which RNA polymerase is involved^{15–18}. Similarly, muscle actin gene activation occurs several hours after the levels of both newly synthesized and accumulated cytoplasmic poly(A)⁺ RNA begin to increase¹⁹. We conclude that the sarcomeric actin genes are activated at stage 13, independently of any other gene so far identified.

The relatively constant level of the 2.3-kilobase (kb) cytoskeletal actin mRNA detected in all stages before gastrulation could result from *de novo* gene transcription or the inheritance of maternally derived message (or indeed a combination of both). Certainly by neurula stage, any pool of inherited cytoskeletal transcripts is supplemented by newly synthesized mRNA.

Regional actin gene expression

By the early neurula (stage 14), *Xenopus* embryos show morphological differentiation, possessing endoderm, mesoderm and ectoderm germ layers. The future anterior-posterior axis of the embryo is evident from the polarity of the elevated neural plate, formed by dorsal ectoderm; beneath it lies the notochord (the embryonic 'backbone') which segregates as a mid-dorsal strip from the mesoderm layer. On either side of the notochord, the thickened, dorsal regions of mesoderm largely comprise the precursors of embryonic muscle (the somites)²⁰. We dissected neurula embryos (neural folds complete; stage 18) into six different pieces (Fig. 5) and used the RNA from each in the S_1 nuclease protection assay. All the pieces contain cytoskeletal actin mRNA in approximate proportion to their tissue mass (data not shown); α -cardiac and α -skeletal actin mRNA distribution is shown in Fig. 6. Strikingly, the sarcomeric actin transcripts are restricted almost exclusively to the somite region of the mesoderm and are completely absent from an equivalent amount of the adjacent notochord tissue. A minor signal is detected in the ventral ectomesoderm, possibly arising from the

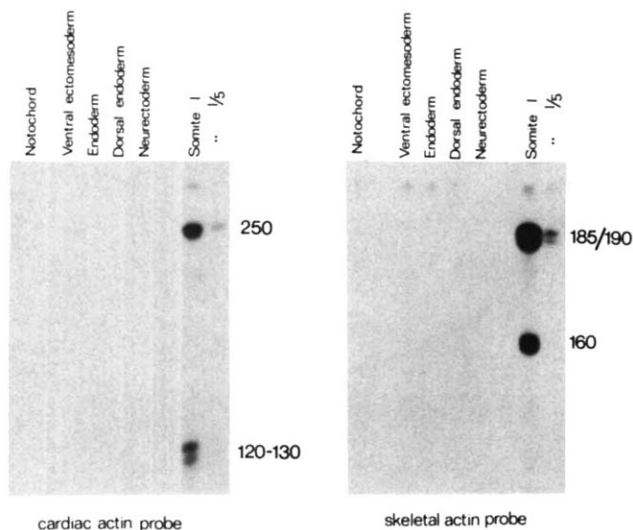


Fig. 6 Region-specific accumulation of muscle actin mRNAs. The six different embryo fragments (Fig. 5) were assayed for the presence of actin mRNAs by the S_1 nuclease protection procedure. α -Cardiac actin mRNA is detected by the presence of protected fragments 250 and 120–130 nucleotides long. α -Skeletal actin mRNA gives a major doublet 185 and 190 nucleotides long (see Fig. 2 legend), and a minor protected fragment about 160 nucleotides in length. This fragment results from partial cleavage of the probe M3 at an A+T-rich region (18 out of 20 bases are A or T). Cleavage in this region is variable, giving a prominent signal in this experiment but a very minor one in others (for example, Fig. 2b).

mesodermal component of this fragment which subsequently gives rise to the embryonic heart.

Muscle-gene activation is thus restricted to the mesoderm cell layer in early embryos. Furthermore, within the mesoderm the expression of these genes is precisely regionalized, because the most dorsal portion, the notochord, contains neither α -cardiac nor α -skeletal actin mRNA. We conclude that sarcomeric actin gene activity is restricted to the embryonic tissue from which muscle cells are later derived. Muscle actin mRNA accumulation commences very early in development, several hours before the morphological differentiation of muscle tissue. Developmental regulation of actin genes has also been studied in fruitflies^{21,22} and sea urchins^{23–25}. Several actin mRNAs are synthesized in these organisms, each with a distinct developmental profile. However, in contrast to amphibians, the appearance of muscle actin gene transcripts is a later embryonic event, coinciding with the formation of muscle-containing tissues in larval or metamorphosis stages.

Myogenesis and actin gene expression

The dorsal, flanking region of the mesoderm is irreversibly committed to embryonic muscle formation in the stage 10 gastrula *Xenopus* embryo²⁶, several hours before newly synthesized muscle proteins are first detected⁷. This may be a common feature of amphibian embryogenesis (see ref. 27). Our present results also show that mesoderm commitment occurs significantly before actin gene transcription and no major store of untranslated muscle actin transcripts is accumulated before α -actin protein is first synthesized in *Xenopus* embryos.

Muscle precursors, the embryonic somites, begin to appear a few hours after sarcomeric actin gene activation⁵. From stage 14 (actin gene activation) to stage 26 (tailbud embryo; 16 somites) the level of muscle actin mRNA increases over 20-fold, yet the total number of the cells in the embryo only doubles. Both somitic mesoderm and subsequent somite cells must therefore accumulate muscle actin transcripts over this period.

During the same interval, more lateral regions of mesoderm extend ventrally, eventually fusing along the midline to form the embryonic heart rudiment, thus being derived from a quite

distinct region of the gastrula mesoderm from the somites. Somitic mesoderm accumulates similar amounts of both α -cardiac and α -skeletal muscle actin mRNA whilst immediately adjacent mesoderm cells and their derivatives (such as notochord) do not. This suggests that the initial events that regulate the actin multi-gene family distinguish muscle actin-encoding genes from their more distantly related cytoskeletal-type counterparts, whilst subsequent controls establish the final tissue specificity of the various muscle actin genes. Vertebrates seem to differ in the extent to which individual sarcomeric actin genes are expressed in each adult, muscle-containing tissue. The predominant muscle actin mRNA in adult mouse heart tissue (the α -cardiac form) provides about one-third of all the sarcomeric actin transcripts in fetal skeletal muscle²⁸. This is replaced by α -skeletal actin mRNA in the skeletal muscle of newborn and adult mice. In neonatal rats, both α -cardiac and α -skeletal actin gene transcripts are present in heart tissue²⁹. In man, both adult heart and skeletal muscle contain a significant proportion of each kind of sarcomeric actin message³⁰. The initial activation of actin genes in early embryos from these species has not been documented.

Co-expression of α -cardiac and α -skeletal actin genes is not a transient phenomenon in early amphibian development, as we have detected roughly equivalent levels of each transcript in embryonic heart, somites and the myotomes of swimming tadpoles (stage 42) 3 days after fertilization (data not shown). It seems highly improbable that only one set of transcripts is utilized in each of these tissues and more likely that each functional embryonic organ uses both types of muscle actin protein in their contractile apparatus. If so, this raises an intriguing problem: why do adult muscle tissues express the sarcomeric actin genes in a tissue-specific manner if such complex regulation is unnecessary in larval stages?

Conclusions

The α -actin genes are the first identified genes known to be activated in a tissue-specific manner in the early amphibian embryo. Transcription of these genes is regulated in a cell type-specific manner, being restricted exclusively to those regions of the mesoderm that are committed to form embryonic muscle. The presence of muscle actin transcripts therefore offers a convenient molecular marker for these cells and their detection provides the basis of an assay for cytoplasmic factors involved in early cell determination³¹.

This work was initiated in the MRC Laboratory of Molecular Biology, Cambridge. We thank Peter Gunning, Rob Maxson and Larry Kedes (Stanford University) for help in the construction of cDNA libraries. T.J.M. is a Royal Society Research Fellow in Biological Sciences. S.B. is the recipient of a USPHS National Research Service Award.

Received 28 March; accepted 9 August 1984.

- Gerhart, J. C. in *Biological Regulation and Development* Vol. 2 (ed. Goldberger, R.) 133–316 (Plenum, New York, 1981).
- Davidson, E. H. *Gene Activity in Early Development* (Academic, New York, 1976).
- Pollard, T. D. & Weihing, R. R. *CRC Crit. Rev. Biochem. Sci.* 2, 1–65 (1974).
- Buckingham, M. E. & Minty, A. J. in *Eucaryotic Genes: their Structure, Activity and Regulation* (eds Maclean, N., Gregory, S. P. & Flavell, R. A.) 365–395 (Butterworths, London, 1983).
- Hamilton, L. J. *Embryol. exp. Morph.* 22, 253–264 (1969).
- Muntz, L. J. *Embryol. exp. Morph.* 33, 757–774 (1975).
- Ballantine, J. E. M., Woodland, H. R. & Sturgess, E. A. J. *Embryol. exp. Morph.* 51, 137–153 (1979).
- Cleveland, D. W. *et al. Cell* 20, 95–105 (1980).
- Vandekerckhove, J. & Weber, K. *FEBS Lett.* 102, 219–222 (1978).
- Vandekerckhove, J. & Weber, K. *Eur. J. Biochem.* 90, 451–462 (1978).
- Vandekerckhove, J. & Weber, K. *Differentiation* 14, 123–133 (1979).
- Formwald, J. A., Kuncio, G., Peng, I. & Ordahl, C. P. *Nucleic Acids Res.* 10, 3861–3976 (1982).
- Kost, T. A., Theodorakis, N. & Hughes, S. H. *Nucleic Acids Res.* 11, 8287–8302 (1983).
- Vandekerckhove, J., Franke, W. W. & Weber, K. *J. molec. Biol.* 152, 413–426 (1981).
- Sargent, T. D. & Dawid, I. B. *Science* 222, 135–139 (1983).
- Woodland, H. R., Flynn, J. M. & Wyllie, A. J. *Cell* 18, 165–171 (1979).
- Newport, J. & Kirschner, M. *Cell* 30, 675–686 (1982).
- Shiokawa, K. *et al. Devl Biol.* 68, 503–514 (1979).
- Sagata, N., Shiokawa, K. & Yamana, K. *Devl Biol.* 77, 431–448 (1980).
- Nieuwkoop, P. D. & Faber, J. *Normal Table of Xenopus laevis* (North Holland, Amsterdam, 1967).
- Sanchez, F., Tobin, S. L., Rdest, U., Zulauf, E. & McCarthy, B. J. *J. molec. Biol.* 163, 533–551 (1983).

22. Fyrberg, E. A., Mahaffey, J., Bond, B. J. & Davidson, N. *Cell* **33**, 115-123 (1983).
23. Scheller, R. H. *et al. Molec. cell. Biol.* **1**, 609-628 (1981).
24. Shott, R. J., Lee, J. J., Britten, R. J. & Davidson, E. H. *Dev. Biol.* **101**, 295-306 (1984).
25. Crain, W. R., Durica, D. S., Cooper, A. D., Van Doren, K. & Bushman, F. D. in *Muscle Development: Molecular and Cellular Control* (eds Pearson, M. & Epstein, H. F.) 97-105 (Cold Spring Harbor Laboratory, New York, 1982).
26. Slack, J. M. W. & Forman, D. *J. Embryol. exp. Morph.* **56**, 283-299 (1980).
27. Mohun, T. J., Tilly, R., Mohun, R. & Slack, J. M. W. *Cell* **22**, 9-15 (1980).
28. Minty, A. J., Alonso, S., Caravatti, M. & Buckingham, M. E. *Cell* **30**, 185-192 (1982).
29. Mayer, Y., Czosnek, H., Zeelon, P. E., Yaffe, D. & Nudel, U. *Nucleic Acids Res.* **12**, 1087-1100 (1984).
30. Gunning, P., Ponte, P., Blau, H. & Kedes, L. *Molec. cell Biol.* **3**, 1783-1791 (1983).
31. Gurdon, J. B., Brennan, S., Fairman, S. & Mohun, T. J. *Cell* (in the press).
32. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
33. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. *J. molec. Biol.* **143**, 161-178 (1980).
34. Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107**, 303-314 (1980).
35. Nasmyth, K. *Nature* **302**, 670-676 (1983).
36. Berk, A. J. & Sharp, P. A. *Cell* **12**, 721-732 (1977).
37. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. *Biochemistry* **16**, 4743-4751 (1977).
38. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual*, 202-203 (Cold Spring Harbor Laboratory, New York, 1983).

Resolution of synthetic *att*-site Holliday structures by the integrase protein of bacteriophage λ

Pei Ling Hsu* & Arthur Landy

Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912, USA

*Site-specific recombination of the bacteriophage λ genome into and out of the host bacterial genome is postulated to involve the formation of Holliday structure intermediates by reciprocal single-strand exchanges. Synthetic analogues of the predicted recombination intermediates are resolved in vitro by the protein product of the λ *int* gene. Some of the structural features and reaction conditions for this genetic recombination can now be defined.*

INTEGRATION of the bacteriophage λ chromosome into a bacterial chromosome occurs by a reciprocal site-specific recombination between the phage *attP* site and the bacterial *attB* site. The resulting prophage *att* sites, *attL* and *attR*, are themselves able to recombine to regenerate *attP* and *attB* during excision of the viral genome (see refs 1, 2 for reviews). Both integrative and excisive recombination require the phage-encoded protein *Int* and the host-encoded protein IHF (integration host factor)^{3,4}. Additionally, efficient excision requires the phage-encoded protein *Xis*^{5,6}. Although purified *Int* protein is a type I topoisomerase that nicks and reseals pBR322 supercoiled DNA one strand at a time³, no activity other than specific DNA binding has been found for purified IHF^{1,4}.

The *attP* site has 240 base pairs (bp)⁷; it contains a 15 bp 'common core' sequence (occurring in all four *att* sites)⁸, three binding sites for IHF¹ and two classes of *Int* binding sites^{9,10}. Essential DNA sequences to the left or right of the central 'core region' comprise the P arm and P' arm respectively. (Similarly, in *attB* the essential left and right sequences are labelled B and B' respectively.) The *attB* site is much smaller, extending approximately 5 bp to either side of the 15 bp common core¹¹; it does not have any IHF recognition or arm-type *Int* binding sites. *attB* and *attP* do, however, have a similar junction-type *Int* binding site at each core-arm junction¹⁰.

Int-dependent recombination does not involve any degradation or synthesis of DNA nor does it require any high-energy cofactors⁴. During recombination, the DNA strands are cut within the common core at position -3/-2 (from the centre of the core) in the 'top' strand and at position +4/+5 in the 'bottom' strand, to generate a 3'-protruding 7 bp stagger or overlap region¹², (referred to as O in POP' and BOB'). Within the overlap region, DNA:DNA homology between the *att* sites is necessary for efficient recombination¹³. Genetic evidence suggests that at least some fraction of *Int*-dependent recombinants proceed via a single-strand exchange intermediate or Holliday structure^{14,15}.

Holliday structures, also referred to as χ -forms, are formed by the reciprocal exchange of single strands between two DNA duplexes¹⁶⁻¹⁸. They are thought to be intermediates in several recombination pathways, and would be resolved to recombinant products by a second cycle of nicking and re-ligating^{19,20}. χ -

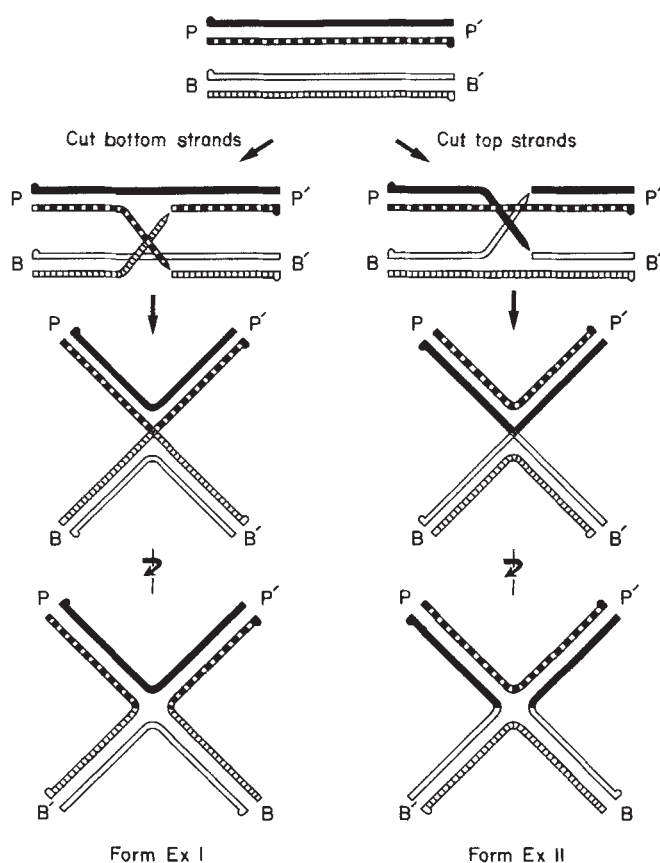
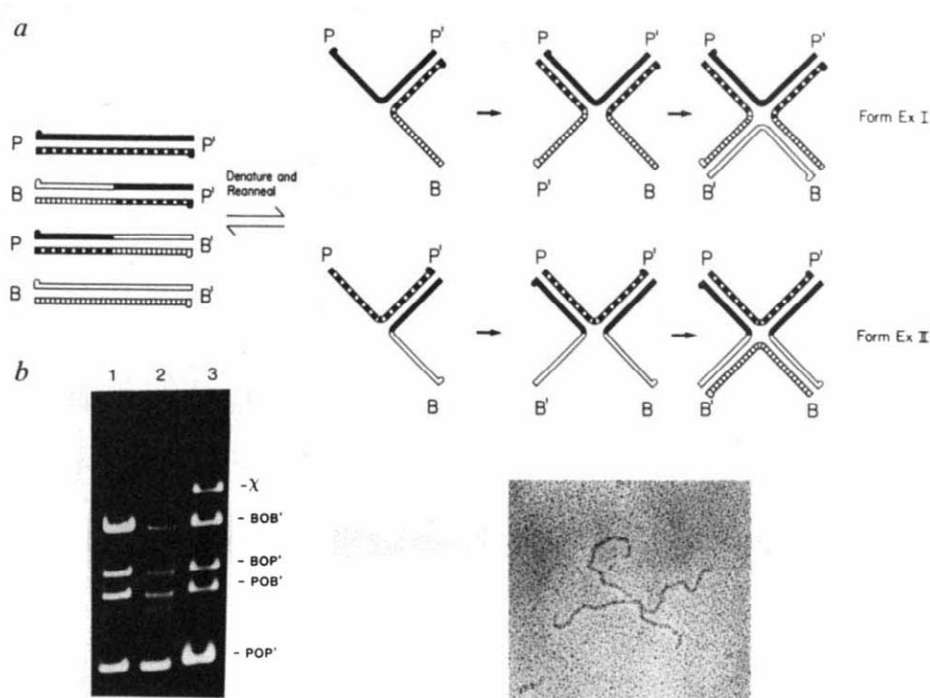


Fig. 1 Formation of Holliday structures (χ -forms) during λ site-specific recombination. The *attP* top (—) and bottom (·····) strands and the *attB* top (—) and bottom (·····) strands have their 5' termini indicated by a knob. The cutting and exchange of two bottom strands (left) results in form ExI, whereas the cutting and exchange of two top strands (right) results in form ExII. In the case where *attL* and *attR* are the parental DNAs (not shown here), the cutting and exchange of the two top strands would result in form ExI and the cutting and exchange of the two bottom strands would result in form ExII.

* Present address: Department of Pathology, University of Texas Health Science Center, Houston, Texas 77225, USA.

Fig. 2 Construction of forms ExI and ExII Holliday structures with a branch point in the common core region of the *att* site. Equivalent amounts of the four parental fragments, containing the POP', BOP', POB' and BOB' DNA sequences, were denatured and reannealed as described here. **a**, Schematic illustration of the stepwise formation of χ -structures during the reannealing process. Designations for the *att*P top (—) and bottom (---) strands and the *att*B top (—) and bottom (---) strands are also used to indicate the corresponding DNA strands in the prophage *att* sites BOP' and POB'. The 5' termini are marked with a knob. **b**, The ethidium bromide-stained agarose gel shows the four DNA restriction fragments before (lane 1) and after (lane 2) denaturation and after reannealing (lane 3). Electron micrograph shows DNA eluted from the gel band marked χ -form.



Methods: 40–120 pmol of restriction fragments in 0.8–1.0 ml were denatured and reannealed by one of the following two procedures: (1), the DNAs were denatured by boiling at 100 °C for 2 min in 1 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, followed by rapid chilling in ice water. One-tenth volume of 20×SSC (3 M NaCl, 0.3 M Na citrate) was added and the mixture was placed in a 65 °C water bath that was then allowed to equilibrate to room temperature overnight. (2), DNA restriction fragments in low melting agarose were heated at 68 °C until the agarose was melted and then placed at 37 °C. They were then denatured in 0.1 M NaOH for 5 min; after adding 1/2 vol of deionized formamide and neutralizing with acetic acid, they were incubated at 37 °C overnight. The χ -forms were purified by gel electrophoresis in 1% agarose at 1.8 V cm⁻¹ in E* buffer (18 mM NaCl, 2 mM EDTA, 40 mM Tris, 20 mM sodium acetate, adjusted to pH 8.0 with acetic acid). DNA was electroeluted from the excised gel band and purified by elution from a BND cellulose column as described previously⁴².

forms have been observed in *Escherichia coli* with phage^{21–24} and plasmid DNAs²⁵, in yeast with 2 μ circle DNA²⁶ and in HeLa cells with adenovirus DNA²⁷. In the Int-dependent pathway of λ it has not yet been possible to detect χ -form recombination intermediates. We report here an investigation of this aspect of the recombination reaction by construction of 'synthetic' Holliday structures that are structurally analogous to a reciprocal single-strand exchange intermediate in site-specific recombination.

Generation of Holliday structures

In forming Holliday structures (Fig. 1), the two DNA helices are first aligned with respect to their common core sequences. The cutting and reciprocal exchange of the two 'bottom' strands results in form ExI, and of the 'top' strands, form ExII. (We identify the individual DNA strands in each helix as either 'top' or 'bottom' in accordance with the convention of writing the four *att* sites as POP', BOB', POB' and BOP'; see Fig. 1.) In site-specific recombination, forms ExI and ExII are not conformational isomers—they cannot be interconverted without breakage and resealing of the DNA strands. This contrasts with Holliday structures generated in homologous recombination, where forms ExI and ExII are indistinguishable because both partners have the same DNA sequence.

We constructed 'artificial' Holliday structures (that is, without recombination) from four restriction fragments containing the POP', BOB', BOP' and POB' DNA sequences. A mixture of the four restriction fragments was denatured and reannealed in a manner analogous to that first used by Bell and Byers²⁶. During renaturation, some of the single strands anneal with their original complement to regenerate the original restriction fragment. Alternatively, annealing between two strands originating from different restriction fragments (such as POP' and POB') generates a 'Y' structure comprising one duplex arm (such as P) and two single-stranded tails (such as P' and B'). Each of the single-

stranded tails can further anneal with complementary sequences originating from a different restriction fragment, and this leads by analogous steps to formation of the four-armed Holliday structure (Fig. 2a).

Forms ExI and ExII comprise two different sets of DNA strands; from each restriction fragment one DNA strand is found only in ExI, for example, the top strand of POP', and the other strand is found only in ExII, for example, the bottom strand of POP' (see Fig. 2a). Thus, by starting with a POP' restriction fragment containing ³²P in only the top strand or the bottom strand, it is possible to label specifically either ExI or ExII respectively. In an alternative procedure it is possible to start with the isolated strands of each restriction fragment; then the appropriate combination of four (out of the total of eight) can be annealed to generate either ExI or ExII.

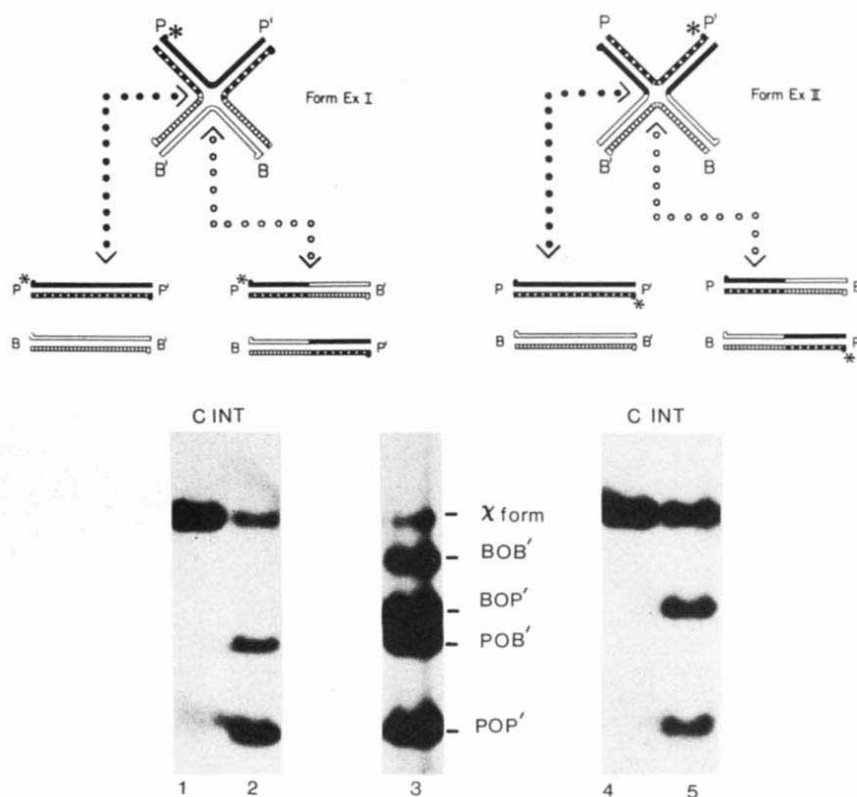
The χ -structure has a higher molecular weight than any of the other DNAs in the annealing mixture and is readily purified by agarose gel electrophoresis on the basis of its unique mobility (Fig. 2b). To prove that the unique slow-migrating band is indeed χ -form DNA, we eluted it from the gel and examined it in the electron microscope. At least 90% of the DNA has the expected χ -structure and arm lengths (see Fig. 2c). Furthermore, if eluted DNA was subjected to a second cycle of denaturation and renaturation, the four initial restriction fragments were recovered as expected. Finally, in the resolution experiments described below we were able to convert 100% of the χ -form DNA preparation into those linear products expected from cutting at the branch points of a simple χ -form structure.

Resolution of Holliday structures

A critical test of whether the synthetic χ -structures do correspond to intermediates of site-specific recombination is the ability of Int protein to resolve them to the expected linear products. Theoretically a synthetic *att* site Holliday structure might be resolved in either the integrative mode, generating *att*L

Fig. 3 Resolution of forms ExI and ExII by Int protein. The strands of the four *att* sites and the 5' termini are designated as in Fig. 2. The positions of the unique ^{32}P label (*) in each χ -form that are expected after resolution are shown in the schematic diagram (see text). Lane 3 shows the positions of the χ -forms and the four restriction fragments used to make the χ -forms. Each labelled χ -form was incubated without (C) or with (INT) Int protein. Radioactive bands were visualized by autoradiography.

Methods: χ -Forms were constructed according to the first procedure described in Fig. 2 legend using the POP' *Hind*III-*Bam*HI-492 fragment of pWR1, the BOB' *Eco*RI-*Bam*HI-1740 fragment of pWR101, the BOP' *Eco*RI-*Bam*HI-1210 fragment of pPH201 and the POB' *Hind*III-*Bam*HI-1020 fragment of pPH202 (Table 1). The POP' restriction fragment was labelled before denaturation with [γ - ^{32}P]ATP and polynucleotide kinase at the *Hind*III site (for labelling form ExI) or at the *Bam*HI site (for labelling form ExII). Each reaction mixture (20 μl) contained 0.1 μg of ^{32}P -labelled form ExI or form ExII in 50 mM Tris-HCl (pH 7.9), 5 mM EDTA, 50 mM KCl and 6.25 mM spermidine. Incubation with 1 U of Int protein (purified by N. Hasan) was carried out at 25 $^{\circ}\text{C}$ for 60 min. (In these reaction conditions 1 U of Int plus 1 U of purified IHF^{4,43} gives optimal recombination of 0.1 pmol supercoiled *att*P, pWR1, and 0.1 pmol of linear *att*B, pWR101.) The reactions were terminated with SDS (0.1% final) and electrophoresed on a 1% agarose gel as described for Fig. 2.



and *att*R (vertical cuts in ExI and ExII, Fig. 3) or in the excisive mode, generating *att*P and *att*B (horizontal cuts in ExI and ExII, Fig. 3). This resolution reaction is presumably equivalent to the second half of normal integrative or excisive recombination. In fact, in reaction conditions suitable for *in vitro* recombination, purified Int protein efficiently resolves both forms ExI and ExII into the expected linear products (Fig. 3).

Each χ -form is labelled with only one ^{32}P atom and, therefore, in each pair of products, only one will be radioactively labelled. Under these experimental conditions the resolution of form ExI yields approximately three times more *att*P than *att*R product, thus indicating a preference for the 'excisive mode' (cutting the bottom strands). On the other hand, resolution of form ExII yields approximately twice as much *att*L as *att*P product, thus indicating a preference for the 'integrative mode' (cutting the bottom strands). This is a good example of how an overall preference for cutting the bottom strand has opposite con-

sequences for ExI (generating excision-type products) and ExII (generating integration-type products). The patterns and degree of preferential strand-cutting are significantly influenced by several experimental parameters (currently under investigation); this is a particularly interesting aspect of the reaction because of its relevance to directionality in site-specific recombination.

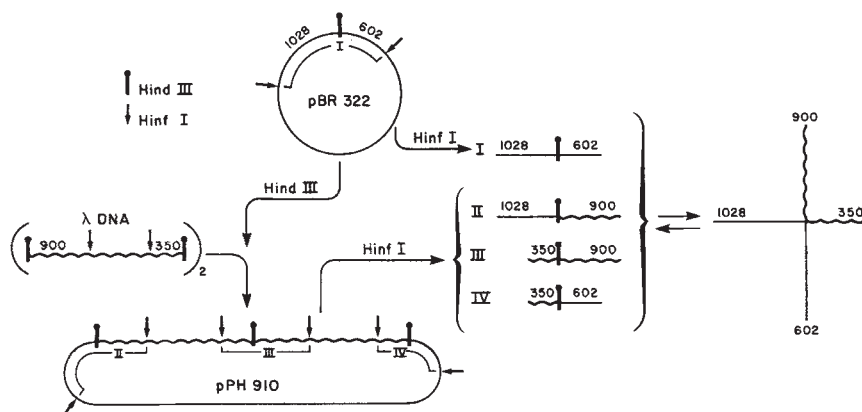
Following resolution, there is an additional band on alkaline denaturing gels, corresponding to full-length *att*R for ExI, and full-length *att*L for ExII (data not shown). This transfer of label from a POP' strand to the corresponding strand of a prophage *att* site indicates the successful ligation of DNA strands during resolution.

Specificity and factors

The strand exchange in λ site-specific recombination resembles the relaxation of superhelical DNA by certain topoisomerases (including the type I topoisomerase activity of Int protein) in

Fig. 4 Construction of a non-*att* Holliday structure. The *Hind*III fragment of λ DNA (—), which extends from 57.0% to 52.4% on the λ map and does not contain an *att* site, was cloned as a tandem duplication into the *Hind*III site of pBR322 (—), to generate plasmid pPH910. Three *Hinf*I fragments of pPH910 were isolated for the χ -form construction: fragment II consists of the left pBR322- λ junction, fragment III of the head-to-tail junction of the two λ fragments, and fragment IV of the right λ -pBR322 junction. Fragment I is the pBR322 *Hinf*I fragment into which the λ DNA had been inserted. For each fragment, and for each arm of the χ -structure, the amount (base pairs) of pBR322 DNA or λ DNA is indicated.

Methods: The χ -form was generated by alkali denaturation and renaturation according to scheme (2) and purified by agarose gel electrophoresis as described in Fig. 2 legend.



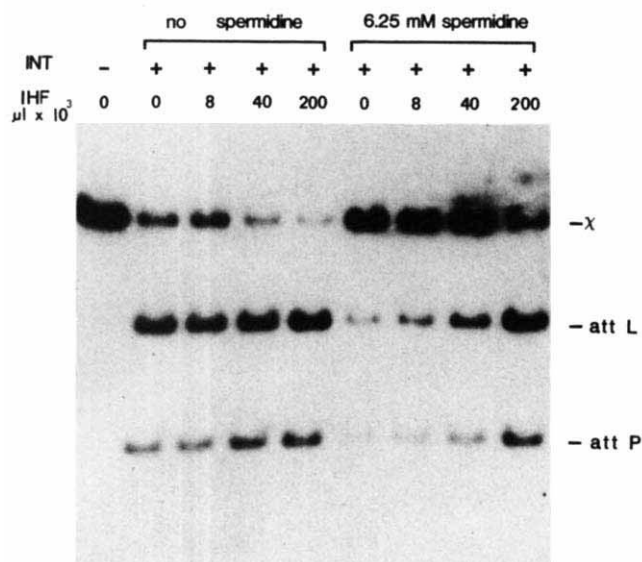


Fig. 5 Effect of IHF protein and spermidine on resolution. ^{32}P -labelled form ExII was constructed and isolated as described in Fig. 3 legend. The composition and processing of each resolution reaction was the same as described for Fig. 3 except that incubation with 0.2 U of purified Int protein was for 30 min. Purified IHF protein⁴ (a gift from H. Nash) at $1 \text{ U } \mu\text{l}^{-1}$ and spermidine were present as indicated.

that the DNA strands are broken and resealed in the absence of DNA synthesis and energy cofactors (see refs 28, 29 for reviews). This raises two points of interest: as the topoisomerase I activity of Int works efficiently with a substrate such as supercoiled pBR322 DNA³, can it resolve a Holliday structure that does not contain an *att* site? Similarly, is the Holliday structure resolved by other DNA topoisomerases?

To address these questions, we constructed a Holliday structure that bears no *att* site DNA sequences (Fig. 4). This structure is not resolved into linear products by Int protein, either in conditions for *in vitro* recombination or conditions for the nicking-closing reaction. The addition of host factor also has no effect. We also tested the ability of three different topoisomerases to resolve the *att* site Holliday structure (these enzymes were a gift from Leroy Liu), HeLa cell topoisomerase I²⁹, T4 topoisomerase II^{30,31} and calf thymus topoisomerase II, whose purification and properties (L. F. Liu, unpublished results) are identical to HeLa topoisomerase II³². In conditions where these enzymes relaxed approximately 30–70% of supercoiled DNA, we observed no resolution of Holliday structure (data not shown). We conclude that, in these experimental conditions, the ability of Int protein to resolve a Holliday structure is specific for the *att* site DNA sequences and is not merely a general property of DNA topoisomerases.

In an *in vitro* reaction, the integrative and excisive recombination functions of Int require the *E. coli*-encoded IHF protein and are strongly stimulated by the presence of spermidine. In contrast, the topoisomerase activity of Int does not require IHF protein and is inhibited by the addition of spermidine³. Int resolution of the Holliday structures is also independent of IHF

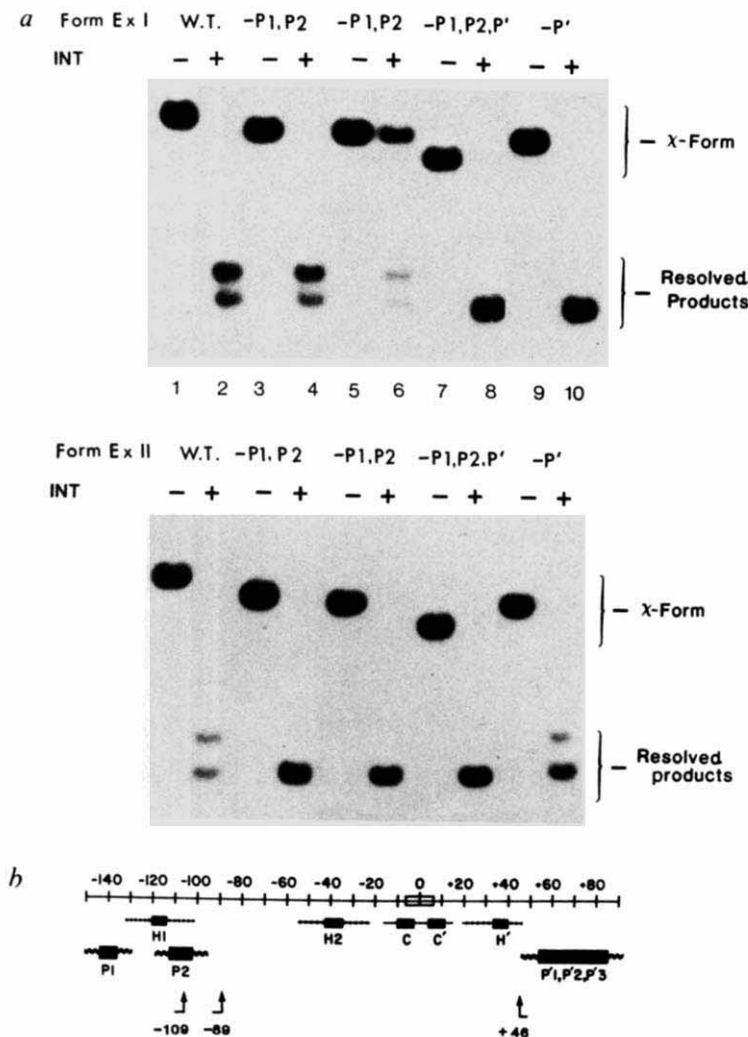


Fig. 6 Int resolution of χ -forms lacking one or more arm-type Int binding sites. χ -forms composed of intact *att* sites (W.T.) or lacking the indicated arm-type Int binding site(s) (–P1, P2, etc.) were constructed from the appropriate *HincII* restriction fragments (Table 1, using the second procedure in Fig. 2). *b*, The *attP* coordinates for the 15 bp common core (—■—); the consensus recognition sequences (—■—) and the approximate region of DNA covered (as determined by nuclease protection) for each of the following: the junction-type Int binding sites, C and C' (—■—), the arm-type Int binding sites P1, P2, P1', P2', and P3 (—■—)^{9,10}, the IHF binding sites H1, H2 and H' (—■—)¹. The endpoints of *att* DNA in the three deletions used to construct the different χ -structures are indicated (–109, –89 and +46) (see Table 1). Each χ -form was incubated in the absence (–) or presence (+) of purified Int protein in a 20- μl reaction that contained $\sim 0.01 \mu\text{g}$ of χ -form, $0.5 \mu\text{g}$ of supercoiled pBR322 DNA and 1 U of purified Int. After electrophoresis of the reaction products, gels were stained with ethidium bromide ($0.5 \mu\text{g ml}^{-1}$) to visualize the nicking-closing activity of Int on the supercoiled pBR322 DNA. The expected levels of activity were observed in each case (data not shown). The ^{32}P -labelled χ -forms and resolution products were visualized by autoradiography. The size (in base pairs) of each labelled resolution product is as follows: BB', 940; BP', 1,077; BD', 886; PB', 1,114; $\Delta\text{B}'$, 972 (lane 4) and 952 (lanes 6 and 8). In this exposure of the autoradiogram, resolution products of similar size are not clearly resolved in several of the lanes; shorter exposures (and other gels) show that both resolution products are obtained in each reaction. The incomplete resolution observed in lane 6 of the upper gel is not typical; in other experiments in these conditions resolution is complete.

Methods: Reaction conditions, processing and gel electrophoresis were as for Fig. 3, with no spermidine. For ^{32}P labelling of forms ExI and ExII, the BOB' *HincII* fragment was first labelled at the 5' termini with polynucleotide kinase and then strand-separated by gel electrophoresis. The top strand of BOB' was incorporated into form ExI and the bottom strand into form ExII (see Fig. 2) by annealing with the other three restriction fragments (Table 1). The specific activity is the same for the entire form ExI family (or the entire form ExII family), because the ^{32}P label is always on the BOB' strand which is common to all five χ -forms (see Table 1).

protein (Fig. 3), resolving 95–100% of the Holliday structure to linear products in the absence of IHF. Int purified from the *himA* deletion mutant K5242 (ref. 33 and H. Miller, in preparation) is also efficient in resolution (data not shown). However, when limiting amounts of Int are used, IHF does stimulate resolution in a dose-dependent manner (Fig. 5). Spermidine inhibits resolution; omission of spermidine results in a three- to fivefold enhancement (Fig. 5). Thus, as might be expected, the requirements for the overall recombination reaction are greater than the requirements for the resolution phase.

Structural requirements

We have shown that the arm-type Int binding sites are essential for integrative recombination. The role of these sites, which are quite distant from the cross-over locus, is unclear (see Fig. 6, bottom)^{7,9}. Are they required throughout the reaction or only involved in some phases? If the latter, are they required for synapsis between the recombining partners and possibly the initial strand exchange, or are they required for resolution of the recombinational intermediate? To address these questions, we have constructed Holliday structures lacking the P arm and/or P' arm Int binding sites and asked which, if any, of these is an acceptable substrate in the resolution reaction.

Holliday structures containing various deletions of the P and/or P' arms were constructed by annealing a ³²P-labelled separated strand of BOB' with different combinations of the appropriate restriction fragments. Two families of χ -forms were constructed, representing forms ExI and ExII for each deletion analysed (Table 1). The restriction fragments and constructions were designed so that all of the arms in every χ -structure are perfect duplexes over their entire length.

In any one series of experiments (see Fig. 6), all of the χ -forms have the same specific activity. This is because the single ³²P atom in each χ -structure is donated by the same BOB' restriction

fragment which has been end-labelled and strand-separated before being distributed into the respective annealing mixtures. This protocol facilitates the comparison of resolution efficiencies under identical Int:DNA ratios. As an additional (internal) control, supercoiled pBR322 DNA was added to each reaction mixture to monitor the topoisomerase activity of Int.

In a 1-h incubation with χ -form, pBR322 supercoiled DNA and Int (present in a molar ratio of 1:20:30), approximately 70% of the supercoiled molecules are relaxed in all of the reaction mixtures. Concomitantly, Int protein resolves all of the Holliday structures with 95–100% efficiency, irrespective of the presence or absence of the arm-type Int binding sites (Fig. 6). For all Holliday structures in this form ExI family, resolution occurs in both the integrative and excisive modes. Similar results and efficiencies are obtained with the analogous family of form ExII Holliday structures. When the concentration of Int is lowered (χ -form:pBR322: Int at 1:20:10) so that χ -form resolution is reduced to ~30%, the resolution efficiency of Holliday structures with each of the deletions is still the same as the non-deleted form (data not shown). We conclude that the arm-type Int binding sites that are required for integrative recombination are dispensable for the resolution of Holliday structures resembling the cross-over region of recombining *att* sites.

Discussion

Does Int-dependent site-specific recombination proceed by a concerted mechanism in which both DNA strands on each partner are cut at the same time and then exchanged and re-ligated, or does it proceed in two distinct steps where first, only one strand on each partner is exchanged to generate a Holliday structure, and second, the single-strand exchange intermediate is resolved by a second cycle of nicking and re-ligation?

We have shown here that Holliday structures with their branch points in the common core region of the *att* site are efficiently

Table 1 Construction of different χ -form structures

χ -Forms Arm sites deleted					Plasmid	<i>att</i>	P arm or Δ arm	P' arm or Δ' arm	B arm	B' arm
Intact	-P1 -P2	-P1 -P2	-P'	-P1 -P2 -P'						
+					pWR1	PP'	-251(734)	+242(517)		
	+				pPH55	Δ P'	-109(592)	+242(517)		
		+			pPH56	Δ P'	-89(572)	+242(517)		
			+		pPH310	P Δ'	-251(734)	+46(326)		
				+	pPH5610	$\Delta\Delta'$	-89(572)	+46(326)		
+	+	+		+	pPH201	BP'		+242(517)	-560	
			+	+	pPH110	B Δ'		+46(326)	-560	
+			+		pPH202	PB'	-251(734)			+380
	+				pPH551	Δ B'	-109(592)			+380
		+		+	pPH561	Δ B'	-89(572)			+380
+	+	+	+	+	pWR101	BB'			-560	+380

χ -Forms with intact *att* sites or lacking one or more of the arm-type Int binding sites (as labelled), were constructed using the indicated (+) restriction fragments, one from each of the four classes (see Fig. 6). For example, to make a Holliday structure lacking the P1 and P2 sites, an isolated BOB' strand from pWR101 was annealed with the Δ OB'-containing fragment of pPH551, the BOP'-containing fragment of pPH201 and the Δ OP'-containing fragment of pPH55. The plasmids from which the *att* site restriction fragments (*HincII*) are isolated are shown. The construction *att* sites (pWR1, pWR101, pPH201 and pPH202), or *att*P with distal deletions of the P and P' arms (pPH55, pPH56, pPH310), has been described previously⁷. Transfer of the *att*P deletions to the other *att* sites was as follows: Int-dependent *in vitro* recombination was carried out (with an extract containing Xis) on head-to-tail heterodimer plasmids containing Δ OP' and POB'; to make pPH551, the dimer consisted of pPH55 and pPH202, to make pPH561, the dimer consisted of pPH56 and pPH202. After recombination the DNA was digested with *EcoRI*, circularized and transformed into HB101. Plasmids containing the recombinant Δ OB' site were identified by their restriction digestion patterns. For plasmids pPH110 (BO Δ') and pPH5610 ($\Delta\Delta'$) the Δ' arm is the *Dde*-*Bam*HI fragment from pPH310. It was ligated through the *Dde* site in the core to the appropriate *EcoRI*-*Dde* fragment, from pPH201 for the B arm or from pPH56 for the Δ arm. The four *att* site arms are denoted P, P', B and B' as described in the text; any change (deletion) in one of the phage *att* site arms is denoted as Δ (corresponding to P) or Δ' (corresponding to P'). The extent of phage *att* site sequence is indicated in base pairs from the centre of the core extending left (-) or right (+) (using the conventions of ref. 8) (see Fig. 6). The *HincII* restriction fragments chosen for these constructions also contain neighbouring pBR322 DNA, thus making it possible to generate physically longer (fully duplex) arms in the final χ -structure; numbers in parentheses indicate the total amount of DNA (phage DNA plus pBR322 DNA) in that arm of the χ -form. DNA from the region around the bacterial *att* site is indicated in base pairs (approximate) from the centre of the core extending left (-) or right (+)⁸; these numbers also indicate the total amount of DNA in that arm of the χ -form (that is, these arms do not have pBR322 sequences).

resolved by purified Int protein. In a mixed recombination reaction containing both χ -form (0.01 pmol) and *attP* and *attB* (0.1 pmol each), 1 U of Int and IHF give ~30% recombination between the *att* sites and ~90% resolution of the χ -form (data not shown). It seems highly unlikely that this unique substrate would be utilized in such a specific and efficient manner if it were not part of the normal recombination pathway. We infer that Int-dependent recombination most probably proceeds through a single-strand exchange Holliday intermediate. Resolution of this intermediate is probably very closely coupled with its formation, as suggested by the efficiency of χ -form resolution, the low frequency of branch migration observed *in vivo* crosses^{14,15} and the failure to find such forms accumulating during recombination. A mechanism that exchanges DNA strands one pair at a time is compatible with the type I topoisomerase activity of Int, and with the four-stranded DNA structure that has been proposed for synapsis^{3,34}.

The finding that purified Int protein efficiently resolves the Holliday intermediate in the absence of IHF (or Xis) substantiates its role in executing the cutting and ligating reactions of recombination^{3,4,12,35}. Because IHF does not participate directly in the strand exchange reaction (resolution), its obligatory participation in integrative recombination is probably due to a role in the formation of Int-*att* complexes or in the synapsis of two *att* sites.

A striking feature of the Int resolution reaction is its specificity for *att* site DNA—in particular for the Int recognition sites at the core-arm junctions. Holliday structures lacking *att* site sequences are not resolved by Int. Of the known enzymes, only the T4-encoded endonuclease VII has also been shown to nick specifically at the branch points of χ -structures³⁶. In contrast to the Int reaction, however, endonuclease VII is not sequence specific and does not appear to reseat the nicked DNA without the aid of DNA ligase.

We have shown here that resolution is efficient even with a Holliday structure lacking all the Int binding sites in the P and P' arms and containing only the four Int binding sites at the core-arm junctions (Fig. 6). Because a Holliday structure lacking the junction-type sites is not resolved by Int (Fig. 4), we conclude that this family of Int recognition sites (C, C', B and B') is both

necessary and sufficient for cutting and re-ligating DNA. The corollary of this conclusion is that the Int binding sites in the arms, although required for *attP* recombination, are not required for the cutting and re-ligation reaction. This is consistent with the finding that a single core-type Int binding sequence, even in non-*att* DNA, is sufficient for nicking by Int protein (ref. 35 and W. Ross and A.L., unpublished results). We infer that the Int sites in the arms function in synapsis of the recombining sites and/or formation of a presynaptic multimeric complex involving *attP* and the necessary proteins. In this context it is interesting that higher order complexes involving *attP* and approximately 10 Int monomers have been observed in the electron microscope^{37,38} and nuclease digestion patterns^{7,9,39} and topological transformations during recombination^{40,41} suggest a wrapping of DNA around, or along the surface of, Int protein(s).

We have shown here that both forms ExI and ExII can be resolved in the integrative mode (producing *attL* and *attR*) or in the excisive mode (producing *attP* and *attB*). This suggests that in these experimental conditions either form ExI or form ExII could serve as an intermediate of either integrative or excisive recombination. Furthermore, as resolution can occur by cutting either pair of strands, there is the possibility that some attempts at recombination are abortive: if the same pair of strands is used for both the first and second cross-over, the original parents are restored. Thus, the overall efficiency of recombination between any two *att* sites could be determined not only by the formation of Holliday intermediates but also by the directionality of their resolution. In our experiments the relative efficiency of resolution was not the same in both directions, with either form ExI or form ExII. It will be interesting to determine how this directionality is influenced by reaction conditions and the various components of the recombination reaction.

We thank Noaman Hasan for purified Int protein, Howard Nash for purified IHF protein, Leroy Liu for the topoisomerases, David Straney for the electron microscopy and Breck Beyers for helpful advice. Lina Vargas and Shirley Rodrigues provided technical assistance. This work was supported by NIH grant AI13544.

Received 20 April; accepted 3 August 1984.

- Nash, H. A. *Rev. Genet.* **15**, 143-167 (1981).
- Weisberg, R. & Landy, A. in *Lambda II* (eds Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. W.) 211-250 (Cold Spring Harbor Laboratory, New York, 1983).
- Kikuchi, A. & Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3760-3764 (1979).
- Nash, H. A. & Robertson, C. A. *J. biol. Chem.* **256**, 9246-9253 (1981).
- Abremski, K. & Gottesman, S. *J. biol. Chem.* **257**, 9658-9710 (1982).
- Better, M., Wickner, S., Auerback, J., Williams, R. & Echols, H. *Cell* **32**, 161-168 (1983).
- Hsu, P.-L., Ross, W. & Landy, A. *Nature* **285**, 85-91 (1980).
- Landy, A. & Ross, W. *Science* **197**, 1147-1160 (1977).
- Ross, W. & Landy, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7724-7728 (1982).
- Ross, W. & Landy, A. *Cell* **33**, 261-272 (1983).
- Mizuuchi, M. & Mizuuchi, K. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3220-3224 (1980).
- Mizuuchi, K., et al. *Cold Spring Harb. Symp. quant. Biol.* **45**, 429-437 (1981).
- Weisberg, R., Enquist, L., Foeller, C. & Landy, A. *J. molec. Biol.* **170**, 319-342 (1983).
- Enquist, L. W., Nash, H. A. & Weisberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1363-1367 (1979).
- Echols, H. & Green, L. *Genetics* **93**, 297-307 (1979).
- Holliday, R. *Genet. Res.* **5**, 282-304 (1964).
- Sigal, N. & Alberts, B. *J. molec. Biol.* **71**, 789-793 (1972).
- Holliday, R. *Genetics* **78**, 273-287 (1974).
- Dressler, E. & Potter, H. A. *Rev. Biochem.* **51**, 727-761 (1982).
- Stahl, F. *Genetic Recombination. Thinking about it in Phage and Fungi* (Freeman, San Francisco, 1979).
- Thompson, B. et al. *J. molec. Biol.* **91**, 409-419 (1975).

- Benbow, R., Zuccavelli, A. & Sinsheimer, R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 235-239 (1975).
- Valenzuela, M. & Inman, R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3024-3028 (1975).
- Thompson, B. J., Camien, M. N. & Warner, R. C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2299-2303 (1976).
- Potter, H. & Dressler, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3000-3004 (1976).
- Bell, L. & Byers, B. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3445-3449 (1979).
- Wolgemuth, D. J. & Hsu, M. T. *Nature* **287**, 168-171 (1980).
- Cozzarelli, N. R. *Science* **207**, 953-960 (1980).
- Gellert, M. A. *Rev. Biochem.* **50**, 879-910 (1981).
- Liu, L. F. & Miller, K. G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3487-3491 (1981).
- Liu, L. F., Liu, C.-C. & Alberts, B. M. *Nature* **281**, 456-461 (1979).
- Miller, K. G., Liu, L. F. & Englund, P. T. *J. biol. Chem.* **256**, 9334-9339 (1981).
- Miller, H. I. & Friedman, D. I. *Cell* **20**, 711-719 (1980).
- Nash, H. A., Mizuuchi, K., Enquist, L. W. & Weisberg, R. A. *Cold Spring Harb. Symp. quant. Biol.* **45**, 417-428 (1981).
- Craig, N. & Nash, H. *Cell* **35**, 795-803 (1983).
- Mizuuchi, K., Kemper, B., Hays, J. & Weisberg, R. *Cell* **29**, 357-365 (1982).
- Hamilton, P., Yuan, R. & Kikuchi, Y. *J. molec. Biol.* **152**, 163-170 (1981).
- Better, M., Chi, L., Williams, R. C. & Echols, H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5837-5841 (1982).
- Davies, R. W., Schreier, P. H., Kotewicz, M. L. & Echols, H. *Nucleic Acids Res.* **7**, 2255-2273 (1979).
- Nash, H. & Pollock, T. *J. molec. Biol.* **170**, 19-38 (1983).
- Pollock, T. & Nash, H. *J. molec. Biol.* **170**, 1-18 (1983).
- Rossi, J. J., Ross, W., Egan, J., Lipman, D. J. & Landy, A. *J. molec. Biol.* **128**, 21-47 (1979).
- Nash, H. A. *Meth. Enzym.* **100**, 210-216 (1983).

Preferential utilization of the most J_H -proximal V_H gene segments in pre-B-cell lines

George D. Yancopoulos*, Stephen V. Desiderio^{†‡}, Michael Paskind^{†‡},
John F. Kearney[§], David Baltimore^{†‡} & Frederick W. Alt*

* Department of Biochemistry and Institute for Cancer Research, Columbia University, College of Physicians and Surgeons, New York 10032, USA

† Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

‡ Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

§ The Cellular Immunobiology Unit of the Tumor Institute, Departments of Microbiology and The Comprehensive Cancer Care Center, University of Alabama, Birmingham, Alabama 35294, USA

The most J_H -proximal V_H gene segments are used highly preferentially to form V_HDJ_H rearrangements in pre-B-cell lines. This result demonstrates that the rate at which immunoglobulin V_H gene segments recombine is influenced by their chromosomal organization, and that the initial repertoire of V_H genes expressed in pre-B cells is strikingly different from that seen in mature populations.

THE genetic information encoding the murine immunoglobulin heavy chain variable (V) region is formed from the juxtaposition of members of three separate clusters of germ-line DNA elements: variable segments (V_H), diversity segments (D) and joining segments (J_H)^{1,2}. Approximately 15 D segments are situated 1 to 80 kilobases (kb) upstream from a 2-kb region containing the four J_H segments³⁻⁵, while the 100–200 V_H segments lie at an unknown distance $5'$ to the D segments^{6,7}. A complete V_HDJ_H variable region gene is assembled during the pre-B-cell stage of the murine B-lymphocyte differentiation pathway via an ordered two-step process: D to J_H joins occur first and on both chromosomes, followed by V_H to DJ_H rearrangement⁶. The tremendous diversity of antibody specificities is due, in part, to the large repertoire of V_H gene segments which are apparently randomly represented in the mature antibody population⁸. The murine V_H gene segments can be divided into approximately ten families based on nucleotide sequence homology; the members of each family are grouped together on the chromosome⁹⁻¹². The relative locations of these families on chromosome 12 have recently been mapped¹³. Little is known about the developmental control of the rearrangement, and subsequent expression, of the many V_H regions. If the choice of V_H gene segments used in rearrangements were not random, it could be mediated by factors such as chromosomal location or recombination recognition sequence elements.

Transformation of murine fetal liver or adult bone marrow cells by Abelson murine leukaemia virus (A-MuLV) generates permanent cell lines which represent the pre-B stage of B-lymphocyte differentiation^{6,14}. Fetal liver-derived transformants generally represent the earliest defined pre-B cell: null(non-immunoglobulin producing) cells which initially contain DJ_H complexes on both chromosomes, but which actively assemble complete V_HDJ_H complexes when propagated in culture⁶. Such cell lines will subsequently be referred to as V_H to DJ_H rearranging. The V_H to DJ_H joins which occur in such lines can be identified and isolated from appropriate cellular subclones^{6,15,16}. Transformants isolated from bone marrow usually represent more mature pre-B cells which contain V_HDJ_H complexes on

at least one allele upon isolation, but do not yet express light chain or have surface immunoglobulin^{6,14}. The ability to study rearrangement outside of the animal, and in cells which lack surface immunoglobulin (sIg) upon isolation, provides a unique opportunity to examine the initial V_H usage pattern in the absence of antigenic or immunoregulatory selective forces acting *in vivo*. Thus, examination of the V_H segments used by such lines may identify developmentally important V_H rearrangement patterns that have not been observed previously.

Two V_H to DJ_H rearranging lines

To explore the possibility of a developmentally-related pattern of V_H segment utilization, we first characterized the V_H segments employed in six independent V_H to DJ_H joins that were identified in subclones of the V_H to DJ_H rearranging lines 22D6 and 40E4^{6,15}. Remarkably, the nucleotide sequence of the V_H portion of these rearrangements (Fig. 1) reveals that three use an identical V_H gene segment designated V_H81X (see sequence $V_H81X(R)$ in Fig. 1; ref. 17), whereas the remaining three rearrangements use V_H gene segments closely related to V_H81X . The V_H81X gene segment and two of the related segments (designated $V_HD6.96$ and $V_HE4.15$, Fig. 1) appear to encode functional V_H regions, and the remaining V_H segment is a pseudogene (designated $V_HE4.Psi$; Fig. 1). These sequences all exhibit stronger nucleotide sequence homology (>80%) to the MOPC21 V_H sequence than to V_H segments from other families (Fig. 1, Table 1), classifying them as members of the relatively small (approximately 10 members) V_H7183 gene family which has $V_HMOPC21$ as its prototype sequence¹². Thus, the cell lines 22D6 and 40E4 use a very limited set of the many V_H segments available.

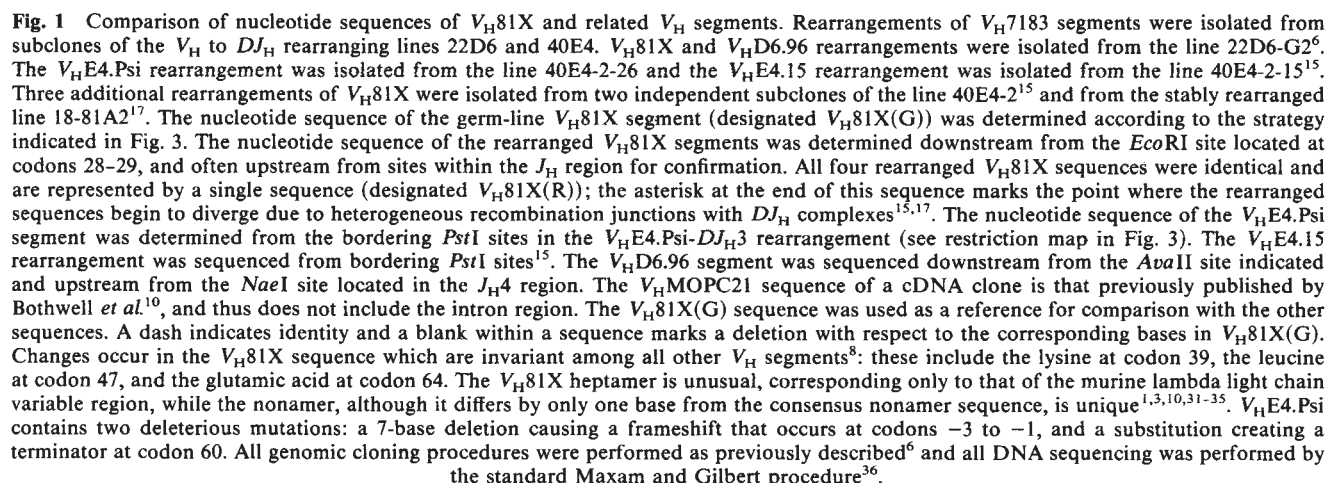
J_H proximity of V_H81X and $V_HE4.Psi$

Because V_H81X was used at a particularly high frequency in the two V_H to DJ_H rearranging lines, it was chosen for further study. *EcoRI*-digested BALB/c liver DNA was assayed for hybridization to probes specific for V_H81X , $V_HD6.96$ and $V_HMOPC21$ (see Fig. 5 for preparation of probes) using the

Table 1 Comparison of % sequence homology in V_H coding regions

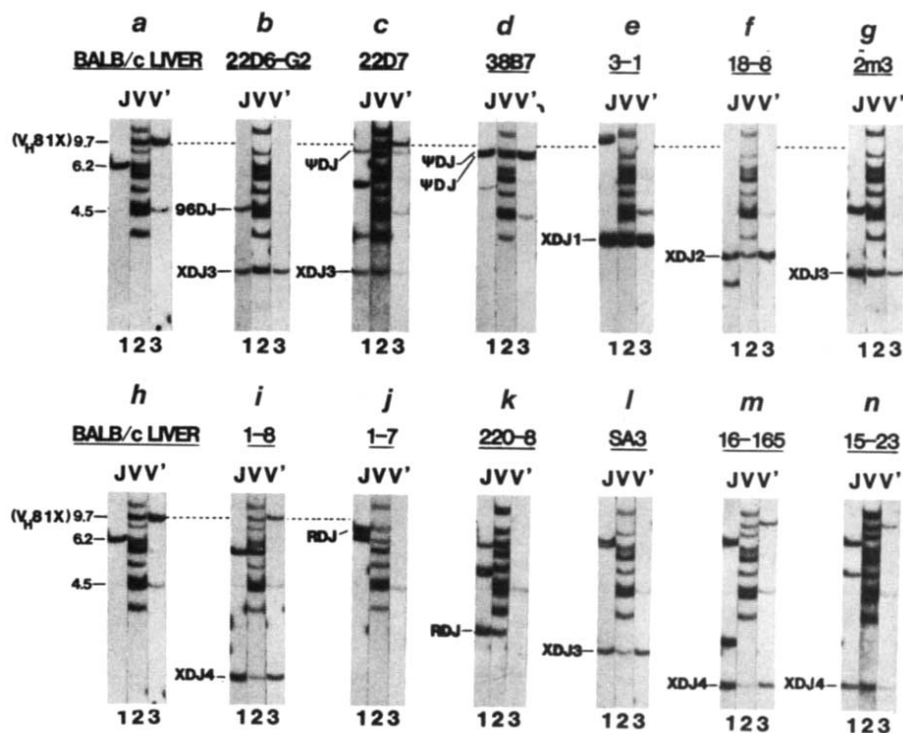
	$V_HD6.96$	$V_HE4.15$	$V_HE4.Psi$	$V_HMOPC21$	V_H141	V_H603	B1-8
V_H81X	83	87	86	81	59	57	55
$V_HD6.96$	—	96	88	85	ND	ND	ND
$V_HE4.15$		—	90	87	ND	ND	ND
$V_HE4.Psi$			—	88	ND	ND	ND

The sequences of V_H81X , $V_HD6.96$, $V_HE4.Psi$ and $V_HE4.15$ are presented in Fig. 1. They are compared within the coding region to each other and to the previously published $V_HMOPC21$ sequence¹⁰. V_H81X is also compared to previously published V_H sequences¹⁰ representative of the three V_H families immediately upstream to the V_H7183 family: V_H141 is representative of the V_HQ52 family, MCPC603 representative of the V_HS107 family, and the B1-8 sequence representative of the V_H558 family. ND indicates that the V_H segments were not compared.



containing this fragment was isolated by screening a BALB/c germ-line DNA library with the V_H81X probe at high stringency (Fig. 3). Analysis of this recombinant clone yielded the sequence of the germ-line V_H81X segment (Fig. 1), and revealed that the 9.7-kb *Eco*RI fragment also contains the unrearranged V_HE4.Psi segment just downstream of the V_H81X segment (Fig. 3). Thus these two V_H segments are closely linked in the germ line.

Fig. 2 Identification of V_H81X and related rearrangements in pre-B-cell lines. The derivation and growth of the A-MuLV-transformed cell lines^{6,14} and the fetal liver hybridomas²⁰ have previously been described; other characteristics of the lines are indicated in Table 2. Approximately 10 μ g DNA from the indicated sources were digested with *Eco*RI, fractionated on 1.0% agarose gels and transferred to nitrocellulose filters; duplicate blots were assayed for hybridization to either 32 P-labelled J_H (see ref. 6) or V_H81X (see Fig. 5) probes. Preparation of high molecular weight genomic DNA, restriction enzyme digests, agarose gel electrophoresis, nick-translations, DNA blotting and hybridizations were performed as described previously¹⁷. BALB/c liver DNA represents the unrearranged (germ-line) state of the immunoglobulin gene segments. The V_H81X filters were first washed at low stringency ($2 \times$ SSC, 68 °C) and then at high stringency ($0.08 \times$ SSC, 68 °C). The sizes of *Eco*RI fragments containing V_H81X and $V_H E4.Psi$ rearranged to each of the J_H segments can be predicted from the germ-line restriction maps of the respective regions (see Fig. 3 for the germ-line $V_H81X/V_H E4.Psi$ restriction map; ref. 2 contains the restriction map of the germ-line J_H region): for V_H81X the predicted sizes are 2.6, 2.3, 2.0 and 1.5 kb; for $V_H E4.Psi$ the sizes are all greater than 7.8 kb; thus, a $V_H DJ_H$ rearrangement hybridizing to the V_H81X probe at high stringency can be classified as a $V_H81X-DJ_H$ or $V_H E4.Psi-DJ_H$ rearrangement based on its size. $V_H DJ_H$ rearrangements in which the V_H segment utilized has been classified as a V_H7183 family member are indicated on the figure (XDJ indicates a V_H81X rearrangement, ΨDJ a $V_H E4.Psi$ rearrangement, 96DJ a $V_H D6.96$ rearrangement, and RDJ indicates that another member of the V_H7183 family was used in the rearrangement). Sizes indicated to the left of the figure are in kilobases. Lane 1: J_H probe (J); lane 2: V_H81X probe, low stringency (V); lane 3: V_H81X probe, high stringency (V').



The V_H7183 family has recently been shown to be the V_H gene family which maps closest to the J_H locus¹³. The line 22D6-G2 has utilized members of the V_H7183 family in $V_H DJ_H$ rearrangements on both chromosomes (V_H81X and $V_H D6.96$; legend to Fig. 1). This line maintains at least one copy of all germ-line *Eco*RI fragments containing V_H7183 family members except for the 9.7-kb fragment which encodes V_H81X and $V_H E4.Psi$ (compare Fig. 2a and b, lane 2). Because V_H to DJ_H joining occurs by a deletional mechanism (for example, ref. 6), this result implies that $V_H81X/V_H E4.Psi$ are located 3' to all other members of the V_H7183 family, and thus are the most J_H -proximal V_H segments yet characterized. This result was confirmed by analysis of many lines with two $V_H DJ_H$ rearrangements: if at least one of the rearrangements utilized V_H81X or $V_H E4.Psi$ (see below), the 9.7-kb germ-line *Eco*RI fragment containing these V_H segments is exclusively deleted (for example, Fig. 2d-g, l).

Utilization of J_H -proximal V_H segments

To determine whether preferential utilization of V_H81X and other members of the most J_H -proximal V_H family was characteristic of the pre-B-cell stage, we devised a simple hybridization assay to examine the $V_H DJ_H$ rearrangements in a large number of lines. The four J_H segments lie within a single, germ-line 6.2-kb *Eco*RI fragment which hybridizes to a J_H probe prepared from the most 3' portion of this germ-line fragment (Fig. 2a, lane 1). Assembly of a $V_H DJ_H$ complex replaces the 5' end of this germ-line J_H fragment, generating a novel *Eco*RI fragment which retains hybridization to the J_H probe, but which now also hybridizes to a probe homologous to the V_H segment utilized in the rearrangement (for example, Fig. 2f, top rearrangement). Under low stringency hybridization conditions, J_H -associated rearrangements utilizing any members of the V_H7183 family will hybridize with a V_H81X probe. Under high stringency condi-

tions, only J_H -associated rearrangements of V_H81X itself will hybridize to the V_H81X probe; these rearrangements include $V_H E4.Psi-DJ_H$ as well as $V_H81X-DJ_H$ rearrangements because an *Eco*RI fragment containing a $V_H E4.Psi-DJ_H$ rearrangement will retain an unrearranged V_H81X segment at its 5' end (see Fig. 3). $V_H81X-DJ_H$ and $V_H E4.Psi-DJ_H$ rearrangements can, however, be differentiated according to the size of the *Eco*RI fragment bearing the rearrangement (see Fig. 2 legend). Any $V_H DJ_H$ rearrangements not hybridizing to a V_H81X probe at low stringency must utilize V_H segments from more 5' families. Because the organization of V_H segments has been most extensively characterized in BALB/c mice, all of the studies described below employed lines of BALB/c origin, except as noted.

Three V_H to DJ_H rearranging A-MuLV transformants were analysed: 22D6, 40E4 and 22D7. The rearrangements isolated from subclones of 22D6 and 40E4 have been described above (representative subclone 22D6-G2 shown in Fig. 2b). The third V_H to DJ_H rearranging line, 22D7, initially contained two DJ_H complexes⁶, but accumulated sub-molar levels of two readily detectable $V_H DJ_H$ rearrangements, one which utilized V_H81X and the other $V_H E4.Psi$ (Fig. 2c). Thus all eight rearrangements characterized in these three V_H to DJ_H rearranging lines utilized gene segments from the V_H7183 family, with half of these rearrangements utilizing V_H81X . Fourteen A-MuLV transformants which had assembled $V_H DJ_H$ rearrangements prior to isolation and which did not undergo further rearrangement during propagation (referred to as stably rearranged) have been assayed as described above. Of the 22 $V_H DJ_H$ rearrangements identified in these lines⁶, at least 10 utilized members of the V_H7183 family, predominantly V_H81X and $V_H E4.Psi$ (Table 2, Fig. 2d-k). Several rearrangements did not use V_H segments which hybridized to the V_H81X probe (for example, the lower rearrangement in Fig. 2f). The V_H segments in five of these rearrangements were characterized by nucleotide sequence

Table 2 V_H segment utilization in the $V_H DJ_H$ rearrangements of pre-B-cell lines

	$V_H DJ_H$ rearrangements	Rearrangements using $V_H 7183$ segments			Rearrangements using segments from more 5' V_H families
	Total	$V_H 81X$	$V_H E4.Psi$	Other	
Fetal liver-derived V_H to DJ_H rearranging lines					
22D6	2	1*	0	1*	0
40E4	4	2*	1*	1*	0
40E1		+		+	—
38B9		+		+	—
38B11		+		+	—
41B1		+		+	+
22D7	2	1	1	0	0
Fetal liver-derived stably rearranged lines					
38B7	2	0	2	0	0
FL	2	1	0	0	1
Marrow-derived stably rearranged lines					
298-13	0	0	0	0	0
220-8	2	0	0	1*	1*
AT1	1	0	0	1	0
2M3	2	1	0	?	?
230-238†	2	0	0	0	2
230-37†	2	0	0	0	2
223-18†	1	0	0	0	1*
18-48	0	0	0	0	0
1-7	2	0	0	1	1*
18-8	2	1*	0	0	1*
1-8	1	1	0	0	0
3-1	2	1	0	0	1
234-8†	2	0	0	0	2
298-8	2	0	0	1	1*
Fetal liver hybridomas BALB/c origin					
15-23	1	1	0	0	0
15-35	0	0	0	0	0
15-56	1	0	0	?	?
15-58	0	0	0	0	0
15-79	1	0	0	?	?
15-83	0	0	0	0	0
16-165	1	1	0	0	0
26-30	1	0	0	?	?
SJL × BALB/c origin					
SA1	1	0	0	?	?
SA2	1	0	0	?	?
SA3	2	1	0	?	?
ACZ	1	0	0	0	1
Totals	43	12	4	6+	14+

The data presented represent a composite of the DNA/RNA hybridization assays and the nucleotide sequence analysis presented in the paper. The number of $V_H DJ_H$ rearrangements in the stably rearranged A-MuLV transformants was previously determined⁶. The number of rearrangements listed for the V_H to DJ_H rearranging lines 40E4 and 22D6 indicates the number of independent $V_H DJ_H$ rearrangements molecularly cloned and characterized from subclones of these lines (see Fig. 1). The V_H to DJ_H rearranging line 22D7 was described in the text. The other V_H to DJ_H rearranging lines were analysed for RNA expression only; the expression data are listed as '+' or '-' under the appropriate column. For the stably rearranged lines, ? in the last two columns indicates that a $V_H DJ_H$ rearrangement co-migrated with a germ-line $V_H 7183$ fragment (for example, upper rearrangement in Fig. 2g), and thus it could not be determined if this rearrangement utilized a $V_H 7183$ segment or a V_H segment from a more 5' V_H family.

* Rearrangements which have been molecularly cloned and sequenced. These include five rearrangements which have utilized segments from families 5' to the $V_H 7183$ family (G.D.Y., T. K. Blackwell, K. Kruger, N. E. Kohl, R. dePinho, M. G. Reth & S.W.A., unpublished data): four different V_H segments from the very large $V_H 558$ family were isolated in rearrangements from the lines 220-8, 223-18, 204-1-7 and 298-8 while the V_H segment in the rearrangement isolated from the line 18-8 corresponded to the prototype sequence of a more 5' V_H family, $V_H 36-60$ ¹⁹. The RNA assay (see Fig. 5) revealed that V_H segments from the $V_H Q52$ and $V_H S107$ families were utilized in at most one rearrangement each in the stably rearranged lines.

† Derived from C57L strain, identical in IgH z haplotype to BALB/c¹².

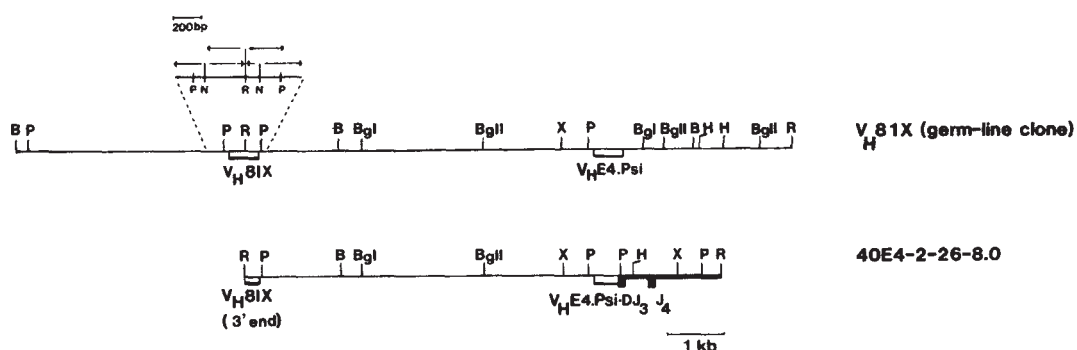
analysis (see Table 2); four were different members of the very large (up to 50 members) $V_H 558$ family, and the other corresponded to the prototype sequence from one of the more 5' V_H families, $V_H 36-60$ ¹⁹.

V_H expression in A-MuLV transformants

Both productive and non-productive rearrangements are expressed at the RNA level in A-MuLV transformed cell lines¹⁷; thus, definition of the V_H -specificity of the expressed RNA can be used to characterize the V_H segments rearranged in these lines. This approach, which can be used to screen efficiently

many lines with several different probes, was used to further characterize V_H segment utilization within the $V_H 7183$ family, and also to examine more readily V_H segment utilization from other families. Poly(A)-containing RNA prepared from 24 A-MuLV-transformed cell lines was slot-blotted onto nitrocellulose filters and assayed for hybridization to probes prepared from six different V_H segments: $V_H 81X$, $V_H D6.96$ and $V_H MOPC21$, which are members of the $V_H 7183$ family (see above); $V_H Q52$ and $V_H S107$, which represent the V_H families immediately upstream to the $V_H 7183$ family^{12,13}; and $V_H 1-7$, a member of the $V_H 558$ family (see legend to Figs 4, 5). Under

Fig. 3 Linkage of V_H E4.Psi and V_H 81X in the germ line. A partial restriction map of a recombinant Charon 4A phage containing a 20-kb germ-line insert, including the 9.7-kb V_H 81X/ V_H E4.Psi-containing *Eco*RI fragment, is presented. This phage was isolated from a library of *Hae*III/*Alu*I partially digested BALB/c sperm DNA³⁷ as described in the text. An enlargement of the region surrounding the V_H 81X segment is depicted above the map with arrows indicating the strategy utilized to obtain the V_H 81X(G) sequence. The map of the cloned V_H E4.Psi rearrangement¹⁵ is presented below the germ-line map, with the restriction maps aligned to demonstrate that the 5' region of the V_H E4.Psi rearrangement is identical to the 5' end of the 9.7-kb V_H 81X-containing germ-line *Eco*RI fragment. The map diverges at the point of recombination of the V_H E4.Psi segment with the DJ_H region (the J_H region is indicated by the bold line). Partial nucleotide sequence analysis also confirmed the identity of the 5' ends of these two clones. The restriction sites are marked as follows: B, *Bam*HI; Bgl, *Bgl*II; BgII, *Bgl*II; H, *Hind*III; N, *Nco*I; P, *Pst*I; R, *Eco*RI; X, *Xba*I.



high stringency conditions these probes strongly hybridize only to identical or very homologous sequences whereas at low stringency they hybridize to additional, less closely related, members of their family (see Fig. 5 legend). Six V_H to DJ_H rearranging lines were assayed for V_H -specific expression. These lines contain subpopulations which have formed complete V_H DJ_H complexes in culture^{6,14,15}. All six lines revealed low levels of expression of RNA sequences which hybridized to V_H 81X and V_H D6.96 but not to V_H MOPC21 (Fig. 4; the RNA sequences which hybridized to the V_H 81X and V_H D6.96 probes were the size of μ mRNA—data not shown). Only one line, 41B1, expressed V_H sequences related to any of the other probes (Fig. 4). These results indicate that the V_H sequences expressed in these lines generally represent a subset of the V_H 7183 sequences that are more closely related to V_H 81X and V_H D6.96 than to V_H MOPC21. Fifteen stably rearranged A-MuLV transformants were assayed for V_H -specific expression (Fig. 5). Ten of these lines expressed V_H sequences from the V_H 7183 family, of which five expressed V_H 81X and five expressed sequences closely related to V_H D6.96 (Fig. 5, Table 2). Tested V_H probes representing families upstream of V_H 7183 were expressed in many fewer lines than were V_H 81X-related segments (Fig. 5, Table 2). This is consistent with the results described above.

In summary, 23 A-MuLV-transformed cell lines of BALB/c origin were analysed for V_H usage by nucleotide sequencing and DNA and/or RNA hybridization studies. The V_H to DJ_H rearranging lines almost exclusively used V_H 7183 gene segments in rearrangements, whereas the stably rearranged lines preferentially utilized V_H 7183 segments, but also used segments from other V_H families (Table 2).

V_H 81X usage in fetal liver hybridomas

To show that preferential use of V_H 81X and related V_H segments was not confined to A-MuLV-transformed cell lines, we examined a set of 12 fetal liver hybridomas that were derived from BALB/c or (BALB $\times$ SJL) F_1 mice²⁰. We identified a total of 10 possible V_H DJ_H rearrangements in these lines by methods described previously⁶ (the remaining J_H rearrangements were DJ_H complexes). Using the genomic DNA hybridization assay described above, we found that V_H 81X was utilized in three of these rearrangements (Fig. 2I–n).

Structure of V_H 81X

Characterization of the V_H segments utilized by pre-B-cell lines allowed identification and nucleotide sequence analysis of five members of the most J_H -proximal V_H family in the BALB/c mouse strain. V_H 81X is the most highly divergent of these five related V_H segments; its sequence is striking in that changes occur at several positions which are invariant among all other V_H genes (see Fig. 1 legend). In particular, the replacement of the highly conserved tryptophan at codon 47 by leucine may disrupt the heavy chain structure and affect binding with light

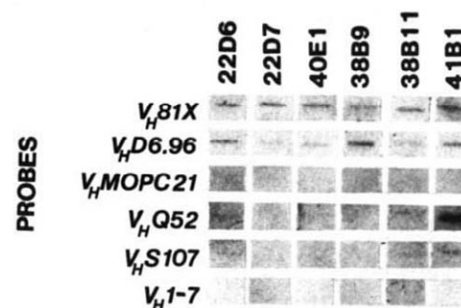


Fig. 4 V_H expression in V_H to DJ_H rearranging A-MuLV transformants. Poly(A)-containing RNA was prepared from the indicated sources as previously described¹⁷; 0.5 μ g of this RNA was denatured in 6% formaldehyde, 50% formamide, 1 $\times$ SSC at 68 $^{\circ}$ C for 10 min, a fourfold excess of 20 $\times$ SSC was added, and the samples were then blotted onto nitrocellulose filters using a slot-blotting apparatus (Schleicher and Schuell-minifold II). The filters were baked, hybridized to probe, and washed as described previously¹⁷. Identical blots were hybridized with the ³²P-labelled V_H -specific probes indicated (V_H 7183 probes described in Fig. 5; the V_H 1–7 segment was prepared from a rearrangement isolated from the A-MuLV transformant 204-1-7 (see Table 2) and is a member of the V_H 558 family; all other V_H -specific probes extensively characterized by G.D.Y. and F.W.A., in preparation). Approximately 1.0 μ g of total RNA prepared from 4-week spleens probed at high stringency served as positive control and is shown in far right in Fig. 5. The exposures depicted were washed at low stringency (1.0 $\times$ SSC, 68 $^{\circ}$ C) except for the blot probed with V_H 1–7 which was washed at high stringency (see Fig. 5). The probe for each set of samples is indicated on the left of the figure.

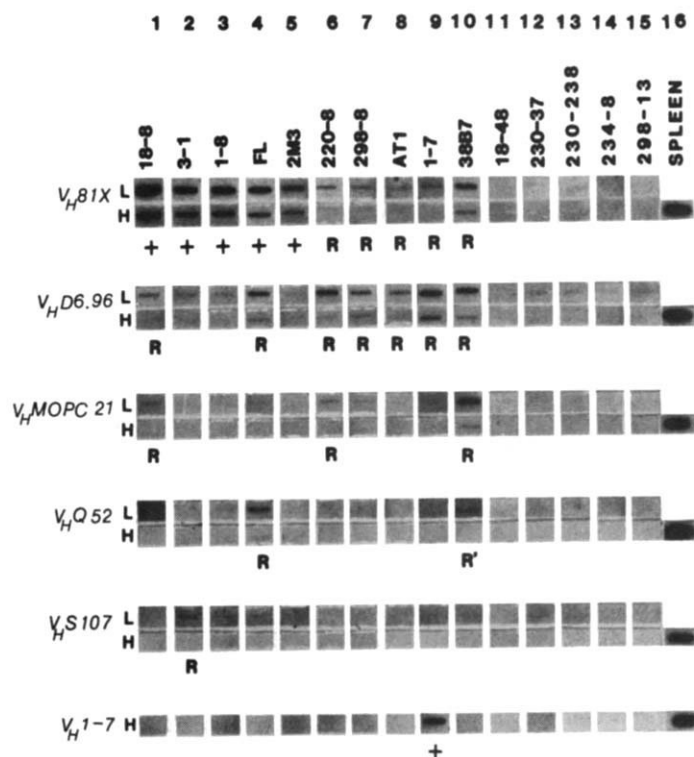
chain (M. Reth, personal communication). The recombination recognition sequences for V_H 81X, which mediate V_H to DJ_H joining, are also quite distinctive (see Fig. 1 legend). It is notable that the V_H 81X segments utilized in the four sequenced V_H 81X- DJ_H rearrangements are identical to the germ-line V_H 81X segment (Fig. 1). This result is consistent with a lack of selection for somatic mutants in the early developmental stages represented by these lines.

Chromosomal location and V_H utilization

Our studies have revealed a striking consistency in the pattern of V_H usage in pre-B-lymphocytic cell lines. A set of the most J_H -proximal V_H segments, typified by V_H 81X, were highly preferentially rearranged in many A-MuLV transformants derived from both fetal liver and adult bone marrow. As discussed earlier, it is unlikely that selection for particular immunoglobulin specificities has influenced our results. It is also unlikely that our results represent an artefact related to the use of permanent cell lines; we have observed this phenomenon in both A-MuLV-

Fig. 5 V_H expression in stably rearranged A-MuLV transformants. Poly(A)-containing RNA was prepared from the indicated lines and processed as in Fig. 4. Exposures of blots washed at low ($1.0\times$ SSC, 68°C) and high ($0.1\times$ SSC, 68°C) stringency, as indicated for each probe, are shown. The blot probed with V_H1-7 was washed only at high stringency; this was because closely related unrearranged V_H gene segments have been found to be expressed in most pre-B-cell lines (G.D.Y. and F.W.A., in preparation). The V_HQ52 probe hybridizes to RNA from the line 38B7 because the V_HQ52 probe hybridizes to $V_HE4.Psi$ sequences at low stringency (G.D.Y. and F.W.A., in preparation), not because of V_HQ52 expression. The hybridization to the V_H81X and $V_HD6.96$ probes has been determined to be of normal μ -size (data not shown). A '+' under a slot indicates that the V_H expressed by the line is completely homologous to the V_H fragment used as a probe, as judged by Southern hybridization data and by maintenance of hybridization even at high stringency. An 'R' under a slot indicates that a line expresses V_H sequences very closely related to the V_H fragment used as probe. An 'R' indicates low-level hybridization that may or may not be due to hybridization within a V_H family. Preparation of probes: The V_H81X fragment was prepared from the germ-line clone by digesting at the *Eco*RI and *Sac*I sites indicated by brackets in the $V_H81X(G)$ sequence (Fig. 1) and purifying the approximately 220-bp fragment spanned by these sites. The $V_HD6.96$ fragment was prepared by digesting at the *Ava*II and *Dde*I sites indicated (Fig. 1) and isolating the intervening fragment. The $V_HMOPC21$ fragment was prepared from a cDNA clone by digesting at the *Dde*I sites indicated in the $V_HMOPC21$ sequence (Fig. 1) and isolating the approximately 200-bp fragment released. All fragments contained V_H coding regions only and spanned both hypervariable regions.

PROBES



transformed pre-B cells and fetal liver (pre-B) hybridomas, and have recently confirmed these findings in studies of normal pre-B cells²¹.

Examples of developmental control related to chromosomal organization are found with mammalian globin genes²², silk moth chorion genes²³, and immunoglobulin genes^{6,24}. Here we demonstrate that members of the most J_H -proximal V_H family (V_H7183) are rearranged preferentially in pre-B-cell lines. Furthermore, there is biased usage within the V_H7183 family: the most J_H -proximal segments ($V_H81X/V_HE4.Psi$) are used most frequently, while a subset of closely related segments (typified by $V_HD6.96$ and $V_HE4.15$) are used more frequently than the remaining members of the family (typified by $V_HMOPC21$). Although fetal liver-derived A-MuLV transformants almost exclusively utilize V_H segments from the V_H7183 family, V_H segments from more 5' families are represented in approximately half the rearrangements made by marrow-derived A-MuLV transformants. Because the fetal liver-derived lines generally represent a less mature stage of pre-B-cell differentiation than marrow-derived lines⁶, this result implies that the repertoire of expressed V_H segments expands during pre-B-cell development. Our data suggests that V_H segments from more 5' families are randomly represented in rearrangements in pre-B-cell lines; many of these rearrangements used different members of the largest V_H family (V_H558), which may contain up to half of the entire V_H repertoire. The simplest mechanism for the preferential rearrangement of V_H7183 segments would involve their relative chromosomal location: for example, potential recombinases may recognize J_H -proximity in the context of one-dimensional scanning⁷. J_H -proximity is not, however, the sole determinant of preferential rearrangement because $V_HE4.Psi$ is located 3' to V_H81X and is used less frequently.

Functional significance of biased V_H usage

The distinctive amino acid sequence encoded by V_H81X has never been found to be expressed in lines representing mature cell types; furthermore, V_H7183 segments are rarely seen in the apparently random representation of V_H segments found in

mature cell types⁸. This reveals a major difference in the repertoire of V_H segments expressed by precursor and mature B-cell populations. The apparently random and extremely diverse set of V_H segments expressed in mature populations could derive from an initial population expressing a biased repertoire by various methods. One such is selection against cells with V_H81X rearrangements occurring during pre-B-cell development; for example, if V_H81X -encoded heavy chains could not associate with light chains, sIg formation would be impossible. Alternatively, even if V_H81X was over-represented in the initial sIg⁺ B-cell population, other clonotypes might be selectively expanded.

Although we have observed preferential V_H7183 segment rearrangement in strains other than BALB/c, in at least one strain V_H81X is not the most J_H -proximal segment (G.D.Y., M. G. Reth and F.W.A., unpublished data). Thus, different V_H segments may be preferentially rearranged in different strains. This preferential rearrangement may simply be a by-product of the recombinational mechanism, or the rearrangement of the most J_H -proximal V_H segments *per se* may be important in early B-cell differentiation, for example, by serving as a substrate for subsequent V-replacement mechanisms. On the other hand, expression of preferentially rearranged segments could play an important role in the developing immune system. Although there are no direct precedents for our results, many studies have suggested that the ability to respond to different antigens follows an ordered developmental sequence²⁵⁻³⁰. It has been shown that highly restricted sets of anti-DNP and anti-TNP antibodies (clonotypes) reproducibly appear during early murine ontogeny³⁰. Recently, Riley and Ogata (personal communication) have cloned the cDNA for one heavy chain involved in an early DNP response. The V_H segment utilized in the variable region coding for this heavy chain is, remarkably, far more homologous to $V_HE4.15$ and $V_HD6.96$ (greater than 95% homology to both) than to any V_H segment previously identified. Thus an antibody made early in development is encoded by a V_H segment which rearranges preferentially in early B-lymphoid cells, suggesting that programmed rearrangement may underlie the programmed appearance of clonotypes.

We thank T. K. Blackwell, M. G. Reth, C. Gee and especially R. Riblett and P. Brodeur for reagents, communication of unpublished results and critically reading this manuscript. This work was supported by American Cancer Society grants NP-393 (F.W.A.) and MV-34M(D.B.), NIH grants AI-29047(F.W.A.),

CA16673 and AI14782(J.F.K.), CA-14051 (a core grant to S. Luria) and an award from the Searle Foundation (to F.W.A.). G. D. Y. was supported by NIH training grant GM-07367, S.V.D. is a fellow of the Jane Coffin Childs Memorial fund and F.W.A. is an Irma T. Hirsch Career Scientist.

Received 30 April; accepted 30 July 1984.

1. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19**, 981-992 (1980).
2. Sakano, H., Kurosawa, Y., Weigert, M. & Tonegawa, S. *Nature* **290**, 562-565 (1981).
3. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676-683 (1980).
4. Kurosawa, Y. & Tonegawa, S. *J. exp. Med.* **155**, 201-218 (1982).
5. Wood, C. & Tonegawa, S. *Proc. natn. Acad. U.S.A.* **80**, 3030-3034 (1983).
6. Alt, F. W. *et al.* *EMBO J.* **3**, 1209-1219 (1984).
7. Tonegawa, S. *Nature* **302**, 575-581 (1983).
8. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. in *Sequences of Proteins of Immunoglobulin Interest* (NIH, Bethesda, 1983).
9. Kemp, D. J. *et al.* *J. molec. appl. Genet.* **1**, 245-261 (1981).
10. Bothwell, A. L. M. *et al.* *Cell* **24**, 625-637 (1981).
11. Loh, D. Y., Bothwell, A. L. M., White-Schaff, M. E., Imanishi-Kari, T. & Baltimore, D. *Cell* **33**, 85-93 (1983).
12. Brodeur, P. & Riblett, R. *Eur. J. Immun.* (in the press).
13. Brodeur, P., Thompson, M. A. & Riblett, R. *UCLA Symp. molec. cell. Biol. new Ser.* **18** (in the press).
14. Alt, F. W., Rosenberg, N., Lewis, S., Thomas, E. & Baltimore, D. *Cell*, **27**, 381-390 (1981).
15. Desiderio, S. V. *et al.* *Nature* (in the press).
16. Yaoita, Y. *Nucleic Acids Res.* **11**, 7303-7316 (1983).

17. Alt, F. W., Rosenberg, N., Enea, V., Siden, E. & Baltimore, D. *Molec. cell. Biol.* **2**, 386-400 (1982).
18. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
19. Near, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 2167-2171 (1984).
20. Burrows, P., LeJeune, M. & Kearney, J. F. *Nature* **280**, 838-840 (1979).
21. Blackwell T. K., Yancopoulos, G. D. & Alt, F. W. *UCLA Symp. molec. cell. Biol. new Ser.* **19** (in the press).
22. Fritsch, E. F., Lawn, R. M. & Maniatis, T. *Cell* **19**, 959-972 (1980).
23. Eickbush, T. H. & Kafatos, E. C. *Cell* **29**, 633-643 (1982).
24. Marcu, K. B. *Cell* **29**, 719-721 (1982).
25. Silverstein, A. M., Uhr, J. W., Kraner, K. L. & Lukes, R. J. *J. exp. Med.* **117**, 799-812 (1963).
26. Sterzl, J. & Silverstein, A. M. *Adv. Immun.* **6**, 337-459 (1967).
27. Ivanyi, J. *Immunology* **28**, 1007-1013 (1975).
28. Lydyard, P. M., Grossi, C. E. & Cooper, M. D. *J. exp. Med.* **144**, 79-97 (1976).
29. Press, J. L. & Klinman, N. P. *Eur. J. Immun.* **4**, 155-159 (1974).
30. Klinman, N. R. & Press, J. L. *Fedn Proc.* **34**, 47-50 (1975).
31. Crews, S., Johanna, G., Huang, H., Calame, K. & Hood, L. *Cell* **25**, 59-66 (1981).
32. Givol, D. *et al.* *Nature* **292**, 426-430 (1981).
33. Ollio, R., Auffray, C., Sikorov, J. L. & Rougeon, F. *Nucleic Acids Res.* **9**, 4099-4109 (1981).
34. Kataoka, T., Nikaide, T., Miyata, T., Moriawaki, K. & Hongo, T. *J. biol. Chem.* **257**, 277-285 (1982).
35. Cohen, J. B. & Givol, D. *EMBO J.* **2**, 1795-1800 (1983).
36. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 419-460 (1980).
37. Davis, M. M. *et al.* *Nature* **283**, 733-739 (1980).

LETTERS TO NATURE

Rapid variability in optical polarization of the quasar-like object OJ287

Anjani K. Kulshrestha, U. C. Joshi & M. R. Deshpande

Physical Research Laboratory, Ahmedabad-380009, India

OJ287 (0851 + 20) is one of a small group of quasar-like objects, the BL Lac group. It has a featureless optical nonthermal continuum, a relatively flat radio spectrum, a high degree of linear polarization at radio and optical wavelengths, it is extremely compact and displays a strong rapid variability at all wavelengths. Rapid variations in the optical flux of OJ287 with a period of about 40 min were observed by Visvanathan and Elliot¹ and by Frohlich *et al.*². At 1.25 μm even more rapid but non-periodic variations were reported by Wolstencroft *et al.*³. We report here observations of rapid and strong variations in the degree of linear polarization of the optical flux of OJ287. We conclude that if the radiation observed from the variable component was of synchrotron nature, then there must be a high degree of relativistic beaming in OJ287. On the other hand, if the variable flux is assumed to be the inverse-Compton scattered flux and relativistic effects are not invoked, OJ287 must have a very compact central source, probably a black-hole accretion-disk system.

The photopolarimetric observations of OJ287 in the ultraviolet, blue, visible, red and infrared (UBVRI) wavebands were made using the Minipol instrument⁴, with GaAs detectors (cooled to dry ice temperatures), on the 1.55-m telescope of the University of Arizona at Mt Catalina on 10-12 January 1984. Normalized Stokes parameters, Q and U , and their errors were calculated from a least squares analysis of the data, and the degree of linear polarization ($P = (Q^2 + U^2)^{1/2}/I$) and position angle ($\theta = \frac{1}{2} \tan^{-1} Q/U$) were calculated from these quantities, where I is the intensity. Sky observations were made approximately every 15 min and the results were obtained from the data corrected for sky background. Instrumental polarization was negligible in comparison with that of the source. Photometric calibrations were made on all nights by observing the star HR382.

Polarization in the R band was monitored from 09:10 to 09:50 UT on 10 January and from 09:55 to 10:50 UT on 11 January (Fig. 1). On 10 January, polarization dropped from 18 to 14% (standard error in polarization, $\sigma_p \approx 0.8\%$) in about 20 min.

During this interval the adjacent sky polarization varied very little (2.7-3.1%, $\sigma_p \approx 1\%$). Two polarization peaks were observed on 11 January; first the degree of polarization dropped from 13% to 9% in about 20 min, and then it increased again to 14% in the next ~15 min. On 12 January, polarization was monitored in I band from 08:50 to 10:00 UT (Fig. 2). Three asymmetric saw-tooth shaped variations were observed. The polarization peaks were separated by about 25 min, and the degree of polarization varied from 12 to 16% ($\sigma_p \approx 0.9\%$). In this interval, four sky observations were made; the sky polarization varied from 1.7 to 2.2%, and the variations in the sky brightness were <0.1 mag. The magnitude, linear polarization, and position angle measurements made in the UBVRI bands on the same date, just after the above-mentioned observations, are shown in Table 1. The observed wavelength-independence of the degree and orientation of polarization indicates that the variations

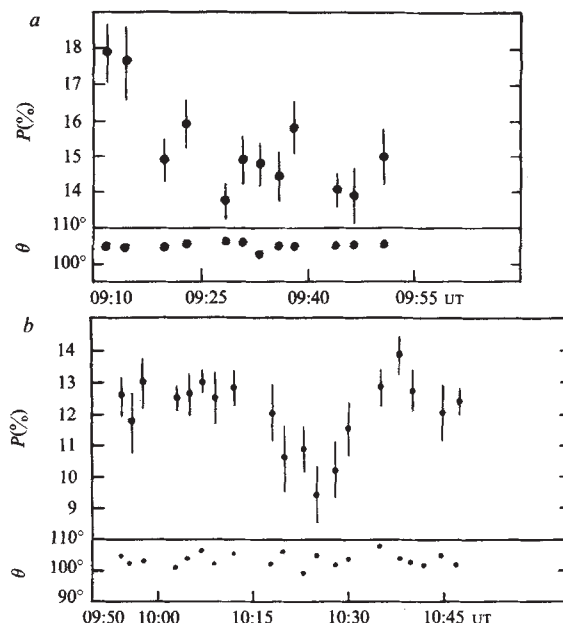


Fig. 1 Time variability of the percentage linear polarization (P) and the position angle (θ) of OJ287 in R band. a, 09:10-09:50 UT on 10 January 1984; b, 09:55-10:50 UT on 11 January 1984. The error bars have a total length of 2 s.d.

Table 1 Photopolarimetric observations of OJ287 on 12 January 1984

Waveband (λ)	Magnitude	P ($\pm\sigma_P$) (%)	θ ($\pm\sigma_\theta$) (deg)
U (0.36 μm)	14.3	13.4 (± 0.8)	97 (± 1.7)
B (0.44 μm)	15.1	13.6 (± 0.6)	94 (± 1.3)
V (0.55 μm)	14.6	13.5 (± 0.4)	93 (± 0.8)
R (0.7 μm)	14.0	14.6 (± 0.8)	94 (± 1.6)
I (0.8 μm)	13.3	13.7 (± 0.4)	94 (± 0.8)

observed in I band were present at least in the whole optical region, that is from U to I bands. The total optical flux, received at Earth, amounts to $2.77 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2}$.

The general trend of the observed variations can be described as follows: the degree of polarization fluctuates by $\sim 4\%$ on a time scale of ~ 25 min; the variations are saw-tooth shaped with the rising edges much steeper compared to the falling ones, and these fluctuations recur at very short time-intervals.

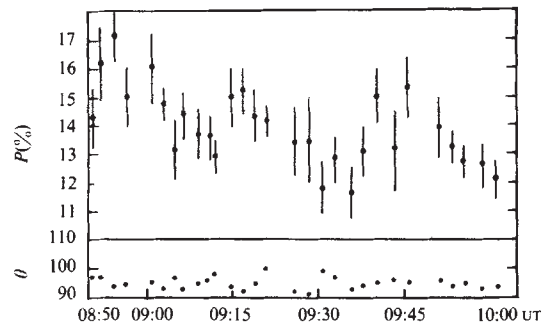
The optical polarization of the star RY Tau was observed on 9 January for ~ 25 min and the degree of polarization was found to lie between 3.2 and 3.3% ($\sigma_p \approx 0.1\%$). The amplitudes of the observed variations in the polarization of OJ287 are very large ($\sim 4\%$) and, thus, these variations cannot be attributed to systematic instrumental effects.

For a source redshift of 0.306 (ref. 5), $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0$, the observed absolute optical luminosity equals $4.8 \times 10^{45} \text{ erg s}^{-1}$. To produce variations in the degree of polarization by as much as 4%, the absolute luminosity of the variable component, L , should be $\geq 2 \times 10^{44} \text{ ergs s}^{-1}$. The observed period of 25 min corresponds to a variability time scale, τ , of ~ 19 min in the rest-frame of the source.

A severe Compton problem (the paradox first described by Hoyle *et al.*⁶) arises if we assume that the variable component was radiating by synchrotron process. This trouble can be removed by invoking bulk relativistic streaming. The requirement that the synchrotron lifetime of electrons be at least equal to the variability time scale gives the value of relativistic Doppler factor: $\delta \geq 15$, where $\delta = (1 - \beta^2)^{-1/2} / (1 - \beta \cos \phi)$ where β is the bulk velocity in units of c , and ϕ is the angle between the direction of motion and the direction along the line of sight. An alternate way of overcoming the Compton problem is to assume that the observed radiation was coming from a very small instability, far from the central source, which derived its energy from the central source (in an unspecified way). Other mechanisms, such as small pitch angle coherent synchrotron emission⁷ or the reacceleration of electrons⁸, could eliminate the Compton problem. However, note that the models which can explain an isolated rapid variation are not necessarily capable of explaining this kind of rapidly recurring variations.

Rees⁹ has shown that rapid periodic variations can be explained using models which invoke relativistic expansion, provided the source is optically thick. An interesting feature of this kind of expanding-source models is that in any outburst, at a given frequency, the source should brighten faster than it fades. This feature would fit to our data when both the variable and the basic synchrotron components of OJ287 were radiating in the same magnetic field configuration.

The other possibility is that the radiation observed from the variable component was inverse-Compton scattered flux from lower frequency photons. The particles which scatter the photons, themselves present some opacity to the photons and thus set a lower limit to the possible variability time scales for an outburst with given luminosity. On the basis of this type of consideration, Fabian¹⁰ has derived a minimum time scale of variation, $\tau \geq L(\text{erg s}^{-1}) / 2 \times 10^{42} \eta$, where η is the efficiency with which matter is converted to energy. Our data imply a value of $\eta \geq 10\%$. For the case of accretion onto massive black hole, such a high value of efficiency is possible only if the accreting material possesses angular momentum¹¹. If we consider the source to be isotropic and free from bulk relativistic motions, and if its luminosity does not significantly exceed the Eddington

**Fig. 2** Time variability of P and θ of OJ287 in I band, 08:50-10:00 UT on 12 January 1984.

limit, due to emission of pulsar-type radiation, then by equating the luminosity of the variable component to Eddington luminosity and taking the appropriate radius of the accretion disk round a black hole to be about five times the Schwarzschild radius, the minimum value of possible variability time scales is ~ 1.3 min which is one-fifteenth of the observed time scale. The super-massive stars would give about 100 times more than this for the minimum time scales. Thus, our observations suggest that the central core of OJ287 is probably a black hole accretion-disk system.

We thank S. P. Pandya and P. V. Kulkarni for encouragement. Tom Gehrels and S. Tapia (Lunar and Planetary Laboratory, University of Arizona) made available Minipol and the telescope. This work was supported by Department of Space, Government of India.

Received 4 May, accepted 15 August 1984.

1. Visvanathan, N. & Elliot, J. L. *Astrophys. J.* **179**, 721-730 (1973).
2. Frohlich, A., Goldsmith, S. & Weistrop, D. *Mon. Not. R. astr. Soc.* **168**, 417-425 (1974).
3. Wolstencroft, R. D., Gilmore, G. & Williams, P. M. *Mon. Not. R. astr. Soc.* **201**, 479-485 (1982).
4. Frecker, J. E. & Serkowski, K. *Appl. Opt.* **15**, 605-606 (1976).
5. Miller, J. S., French, H. B. & Hawley, S. A. *Pittsburgh Conf. On BL Lac Objects* (ed. Wolfe, A. M.) 176-191 (University of Pittsburgh, 1978).
6. Hoyle, F., Burbidge, G. R. & Sargent, W. L. W. *Nature* **209**, 751-753 (1966).
7. Cocke, W. J. & Pacholczyk, A. G. *Nature* **265**, 608-609 (1977).
8. Cavaliere, A. & Morrison, P. *Astrophys. J. Lett.* **238**, L63-L66 (1980).
9. Rees, M. J. *Mon. Not. R. astr. Soc.* **135**, 345-360 (1967).
10. Fabian, A. C. *Proc. R. Soc. A* **366**, 449-459 (1979).
11. Pringle, J. E., Rees, M. J. & Pacholczyk, A. G. *Astr. Astrophys.* **29**, 179-184 (1973).

Outer-core geostrophic flow and secular variation of Earth's geomagnetic field

J. L. Le Mouél

Laboratoire de Géomagnétisme et Paléomagnétisme (ERA 922),
Institut de Physique du Globe, Tour 14, 4 Place Jussieu,
75230 Paris Cedex 05, France

In studies of the temporal variations of the main internal geomagnetic field (the secular variation or SV), it is usual to consider separately the variations of the dipolar and non-dipolar parts which appear to have different time constants. The mechanism that is generally invoked to explain the generation of SV is the advection of the lines of force of the main field by the highly conducting fluid at the top of the core. Such a mechanism involves the main field as a whole and it is not clear *a priori* why its two parts should behave separately. I show here that the Coriolis force will probably dominate the force budget at the top of the core and that, in such a case, the motion of the fluid involves the two parts of the field in a different way; in particular, the existing axial dipolar component is not re-engaged in the process which builds up the SV.

Let us consider the Navier-Stokes equation in the outer layers of the core:

$$\rho(\delta \mathbf{u} / \delta t + \mathbf{u} \cdot \nabla \mathbf{u}) = -\nabla p + \mathbf{J} \times \mathbf{B} - 2\rho(\boldsymbol{\Omega} \times \mathbf{u}) + \rho \nu \Delta^2 \mathbf{u} - \rho \mathbf{g} \quad (1)$$

where ρ is the density, $\mathbf{u}$ the velocity, p the pressure, $\mathbf{J}$ the

electric current density, $\mathbf{B}$ the magnetic field, Ω the Earth's rotation, ν the kinematic viscosity, $\mathbf{n}$ the unit radial vector, g the gravity.

In the frozen-flux approximation, where the core fluid is considered to be a perfect conductor¹⁻⁵, the SV observed outside the core depends only on the (horizontal) motion $\mathbf{u}$ of the fluid at the core-mantle boundary, more exactly below a thin boundary layer. If the flow is assumed to be large scale, its velocity can be estimated from SV data: its order of magnitude U is found to be 10^{-4} m s^{-1} (see ref. 6; the value of $0.2^\circ \text{ yr}^{-1}$ for the westward drift rate corresponds to $4 \times 10^{-4} \text{ m s}^{-1}$). In this large-scale hypothesis, both the Rossby number R and the Ekman number E are quite small. Taking $L = 10^6 \text{ m}$ as a characteristic horizontal length of $\mathbf{u}$, we have $R = U/\Omega L \approx 10^{-6}$ and $E = \nu/\Omega L^2 \ll 1$, whichever value we adopt for the poorly known kinematic viscosity of the core.

As for the time constant of $\mathbf{u}$, the SV field can change significantly—by half its value—in, say, 10 yr (refs 7, 8). But the acceleration term $\rho \partial \mathbf{u} / \partial t$ is fairly negligible ($< 10^{-3}$) compared with the Coriolis force.

Let us now consider the Lorentz force $\mathbf{J} \times \mathbf{B}$. If the field is purely poloidal in the external layers of the core, $B = 5 \times 10^{-4} \text{ T}$, and we get: $|(\mathbf{J} \times \mathbf{B})|/|2\rho(\Omega \times \mathbf{u})| \approx B^2/2\mu\rho\Omega LU \approx 10^{-3}$. It is possible that a toroidal field also exists in the outer layers of the core, as often predicted by dynamo theory, and as is required for electromagnetic core-mantle coupling. But, even if this toroidal field does exist and is intense in the body of the core (up to 10 times the poloidal field), it is certainly less intense in the neighbourhood of the (nearly) insulating mantle. More precisely, let β be the ratio of the dominant part of the horizontal Lorentz force to the horizontal Coriolis force. It is easily shown that $\beta = B_r B_{\text{T}} f(\theta)/(2\mu\rho\Omega LU \cos(\theta))$, where B_r is a typical value of the radial component of the poloidal field at the core-mantle boundary, B_{T} a typical value of the toroidal field B_{T} in the body of the core, l a characteristic vertical scale of B_{T} . $f(\theta)$ depends on the variation of B_{T} with colatitude θ . If, as is generally assumed, B_{T} is antisymmetrical with respect to the Equator and vanishes on the Equator (for example, if B_{T} is a P_2^0 toroidal field due to the differential rotation of the core in the presence of the axial dipolar field), $f(\theta)/\cos(\theta) \approx 1$. When $l = 10^6 \text{ m}$ then β has approximately the same numerical value as B_{T} expressed in tesla, that is to say 5×10^{-2} in the most unfavourable case. If l is smaller, as suggested by Benton and Muth⁹, due to the possibility of a strong gradient of B_{T} near the core-mantle boundary, β is, of course, larger. If not, or if the toroidal field is of the same order of magnitude as the poloidal one, the horizontal projection of equation (1) reduces nearly everywhere to the geostrophic approximation in its usual form:

$$2\rho(\Omega \times \mathbf{u})_{\text{H}} + \nabla_{\text{H}} p = 0 \quad (2)$$

where H indicates horizontal. Equation (2) must be complemented by the radial component of equation (1) whose principal part is the hydrostatic equation. Then

$$\mathbf{u} = (2\rho\Omega \cos \theta)^{-1} \mathbf{n} \times \nabla_{\text{H}} p \quad (\theta \neq \pi/2) \quad (3)$$

Let us now recall an important result due to Backus³. Let B_r be the radial component of the geomagnetic field $\mathbf{B}$ at the core-mantle boundary, $\dot{B}_r$ its time derivative. These quantities are related through the equation

$$\dot{B}_r = \text{div}_{\text{H}}(\mathbf{u} B_r) \quad (4)$$

It follows directly that all motions of the form

$$\mathbf{u} = (B_r)^{-1} (\mathbf{n} \times \nabla_{\text{H}} \psi), \quad (5)$$

where ψ is any well behaved function of longitude ϕ and colatitude θ , are in the so-called null space of $\mathbf{B}$ that is generate no secular variation $\mathbf{B}$ from $\mathbf{B}$.

From equations (5) and (3), the geostrophic motion (3) is in the null space of the axial-dipole field: if, at a certain epoch, the geomagnetic field happens to be that of an axial dipole, it will remain axial dipolar and unchanged.

In general, at the core-mantle boundary,

$$B_r = \alpha_1^0 \cos \theta + \sum_{n,m \neq 1,0} (\alpha_n^m \cos m\phi + \beta_n^m \sin m\phi) P_n^m(\cos \theta)$$

Equation (4) being linear, it follows that the axial dipolar part of $\mathbf{B}$ is not involved in the generation of SV by the geostrophic motion $\mathbf{u}$. Let $\mathbf{B}_1$ denote the axial dipolar component of $\mathbf{B}$, $\mathbf{B}_{\text{nda}}$ the non-dipolar-axial field, that is $(\mathbf{B} - \mathbf{B}_1)$. The behaviour of SV can be summarized by the two equations

$$\begin{aligned} \dot{\mathbf{B}}_1 &= \mathbf{F}_1(\mathbf{u}, \mathbf{B}_{\text{nda}}) \\ \text{and} \\ \dot{\mathbf{B}}_{\text{nda}} &= \mathbf{F}(\mathbf{u}, \mathbf{B}_{\text{nda}}) \end{aligned} \quad (6)$$

The fact that the existing axial dipolar field is not used in the process by which the field is changed provides a simple explanation of why the secular variation of this axial dipolar field (the geological field) does not involve the same time constant as the secular variation of the non-dipole field (all the better since the westward drift of the external layers of the core, which is sometimes considered as the prominent stable part of $\mathbf{u}$, does not generate any $\mathbf{B}_1$ from $\mathbf{B}_{\text{nda}}$).

Let us now assume that the frozen flux approximation still holds for time spans of some thousands of years¹⁰ and consider a reversal of the geomagnetic field: the non-dipole field cannot disappear during the process, otherwise the whole process would stop (equation (6)). Such an extrapolation to long time spans is certainly speculative. But, for time spans short enough for the frozen-flux approximation to hold, a geostrophic flow in the upper layers of the core implies some of the observed features of the secular variation of the geomagnetic field (my arguments in favour seem to fail only if the unobservable toroidal field is both very intense and rapidly varying near the core-mantle boundary).

I acknowledge fruitful discussions with R. Hide and G. Backus. IPGP contribution 788.

Received 25 June; accepted 15 August 1984.

1. Bondi, H. & Gold, T. *Mon. Not. R. astr. Soc.* **110**, 607-611 (1950).
2. Roberts, P. H. & Scott, S. J. *Geomagn. Geoelectr.* **17**, 137-151 (1965).
3. Backus, G. E. *Phil. Trans. R. Soc. A263*, 239-266 (1968).
4. Hide, R. *Phil. Trans. R. Soc. A259*, 615-680 (1966).
5. Voorhies, C. & Benton, E. R. *Geophys. Res. Lett.* **9**, 258-261 (1982).
6. Kahle, A., Vestine, E. H. & Ball, R. J. *Geophys. Res.* **72**, 1095-1108 (1967).
7. Gire, C., Le Mouél, J. L. & Ducruix, J. *Nature* **307**, 349-352 (1984).
8. Courtillot, V. & Le Mouél, J. L. *Nature* **311**, 709-716 (1984).
9. Benton, E. R. & Muth, A. L. *Phys. Earth Planet. Inter.* **20**, 127-133 (1979).
10. Gubbins, D. & Roberts, N. *Geophys. J. R. astr. Soc.* **73**, 675-687 (1983).

Stress corrosion in a borosilicate glass nuclear wasteform

A. E. Ringwood & P. Willis

Research School of Earth Sciences, Australian National University, Canberra 2601, Australia

Most current technologies for the incorporation of high-level nuclear wastes in borosilicate glass wasteforms on a large scale yield products characterized by marked internal stresses developed during cooling and also around crystalline inclusions. We discuss here a typical borosilicate glass wasteform which, when exposed to water vapour and water for limited periods, exhibits evidence of stress corrosion cracking arising from the interaction of polar OH groups with stressed glass surfaces. Glass wasteforms may experience similar stress corrosion cracking when buried in a geological repository and exposed to groundwaters over an extended period. This would increase the effective surface areas available for leaching by groundwater and could decrease the lifetime of

the wasteform. Conventional leach-testing methods are insensitive to the longer-term effects of stress corrosion cracking. We suggest that specific fracture-mechanics tests designed to evaluate susceptibility to stress corrosion cracking should be used when evaluating the wasteforms for high-level nuclear wastes.

The most important property which is used to characterize borosilicate glass wasteforms is their leaching performance in water and aqueous solutions. Most glasses which have been characterized by leach tests have been produced on a small scale in laboratory conditions. Melting is typically carried out under well-controlled conditions above the liquidus, so that there are no crystalline phases present. In many cases, the glasses have been annealed during cooling to eliminate internal stresses. The borosilicate glasses produced by current technologies for the immobilization of high-level nuclear waste (HLW) on a full engineering production scale differ from these 'ideal' laboratory-produced glasses in two important respects. First, they usually contain a few per cent of dispersed crystalline phases, arising either from incomplete solution of the HLW or from crystallization during slow cooling, after the glass has been poured into canisters^{1,2}. Second, when HLW-glasses are cooled in commercial-sized canisters, they are subjected to thermal stresses which cause extensive cracking^{3,4}. Typically, this leads to an increase in the surface area of the glass by about a factor of 10 (ref. 3). Moreover, the remaining domains of uncracked glass are themselves subjected to quite strong internal stress fields.

These characteristics are displayed by a sample of borosilicate glass containing about 25% of simulated HLW produced by the 'in-canister' melting process at the US Department of Energy plant at Savannah River, South Carolina. This technology is the accepted 'back-up' process for the joule-heated ceramic melter on-site. The chemical composition of this glass as determined by electron microprobe analysis is as follows: SiO₂, 46.0; TiO₂, 0.3; Al₂O₃, 4.2; Fe₂O₃, 14.8; MnO, 3.3; NiO, 1.5; CaO, 5.3; Na₂O, 15.2; B₂O₃ + Li₂O, 9.4 wt%. Note, however, that the phenomena discussed here are not specific to glasses of any singular chemical composition, or produced by any one technology, but are generic to HLW glasses produced on a commercial scale by most current technologies because of the residual stresses which these processes induce in the final products.

The sample which was studied (Fig. 1) is bounded by generally conchoidal fracture surfaces. Small impacts readily cause spalling, demonstrating the existence of a pervasive state of internal stress. Numerous crystalline inclusions are visible. Large numbers of sharp cleavage steps are also present (Figs 1, 2). In many cases, cleavage steps propagate outwards from crystalline inclusions (Fig. 2).

Close examination of cleavage steps on surfaces of glass which have been exposed to the atmosphere for ~2 yr show that distinct cracks up to 5 mm long have formed at the base of many of the steps. In contrast, cracks beneath cleavage steps rarely occur on freshly fractured surfaces. The common occurrence of these cracks on surfaces which have been exposed to the atmosphere for an extended period suggests a causal relationship. Related observations and interpretation by Wiederhorn and others⁵⁻⁸ suggests that the cracks have been caused by stress corrosion in the presence of atmospheric moisture. This phenomenon has been widely recognized in stressed glasses in the presence of polar solvents (such as, water) and also in the presence of atmospheric water vapour⁵⁻⁸. The basal corners of the cleavage steps represent sites of localized stress concentrations⁹. The stresses at these sites can be intensified by the pervasive thermal stresses present in the sample, arising from its cooling history. Polar atmospheric water molecules preferentially attack the glass at the sites of greatest stress concentration, causing stress corrosion cracks to develop and propagate⁶⁻⁸.

Several years ago, Wiederhorn⁵ drew attention to the potential importance of stress corrosion on the long-term performance of nuclear-waste glasses, but the significance of his contribution has been largely ignored. Nevertheless, if HLW glass experienced extensive stress-corrosion cracking over an extended period after burial in a geological repository, the effective area

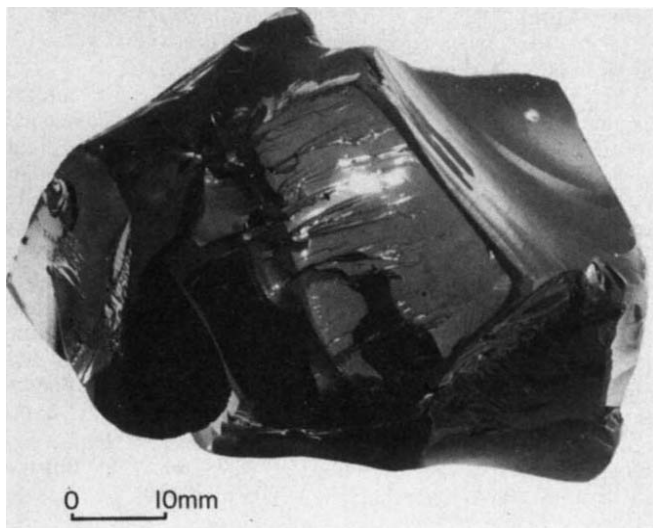


Fig. 1 Sample of borosilicate glass containing ~25% of simulated high-level US defence wastes, produced by in-canister melting at the Savannah River Laboratory of the US Department of Energy. Note the presence of abundant cleavage steps on some of the surfaces.

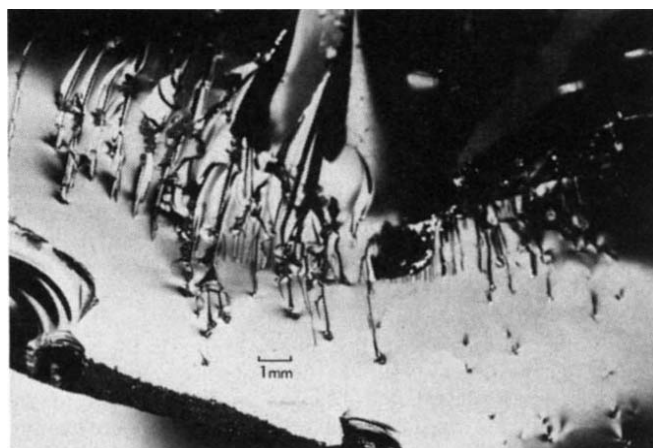


Fig. 2 Glass surface shows complex cleavage steps, mostly originating at crystalline inclusions. Some of these steps represent the outcrops of planar fractures containing iridescent air films.

exposed to leaching would be greatly increased and the survival time of the glass correspondingly decreased. In these essentially unpredictable circumstances, the conventional methods of predicting long-term performance of glasses on the basis of short-term measurements of average leach rates of laboratory-prepared glasses with constant surface area, would be decidedly inadequate.

Studies of polished and thin sections showed that the crystalline inclusions consisted of small spinels (mostly 30–100 µm), commonly sheathed by laths of acmitic pyroxene. The glass immediately surrounding the larger spinel crystals is sometimes observed to be optically anisotropic. This feature is apparently caused by stresses arising from differential thermal expansion coefficients of glass and crystals during cooling.

Samples of glass (dimensions, 1–3 cm) were removed from the interior of the specimen and polished to yield planar surfaces, free of any cracks. The samples were then placed in closed Teflon containers and immersed in 20 ml of deionized water at 95 °C for 25 days. At the conclusions of these treatments, the glasses were covered by loosely adhering iridescent films of hydrous oxides. These were easily removed by a minimal degree

of polishing. The underlying surfaces of the glasses displayed several interesting features (Fig. 3).

The glass immediately surrounding the spinel crystals was selectively removed by leaching so that the spinels were surrounded by deep pits (Fig. 3a). We have already noted that the glass immediately next to some of the spinels is anisotropic, indicative of localized stress. The formation of pits around the spinels is believed to be caused by the higher intrinsic leachability of stressed regions. The samples also displayed systems of intersecting grooves which commonly radiated from, and linked spinel crystals. It seems possible that the development and location of these grooves is determined by the interaction of localized stress fields surrounding spinels and the pervasive state of thermally-induced stress which exists within the glass.

The fact that simple leaching of the smooth surfaces of a typical borosilicate glass causes the rapid development of grooves and pits has important implications for the long term behaviour of these glasses. As noted above, the glass is characterized by a pervasive residual thermal stress. The present experiments show that in the presence of water, grooves and pits develop quite rapidly. The effect of a tensile or shear stress of thermal origin will be to magnify the local stress intensity at the bases of the grooves or pits by a factor proportional to $1/\sqrt{R}$ where R is the local radius of curvature⁷. Thus, the bases of grooves and pits will dissolve more rapidly than plane surfaces and these features will propagate into the interior of the glass much faster than the average rate of surface dissolution. This process of inhomogeneous dissolution may lead ultimately to disintegration of the glass, with a large increase in the effective surface area which can be exposed to the attack of groundwater.

The development of grooves as seen in Fig. 3 seems to reflect a form of stress corrosion. These features may propagate steadily into the interior of the glass through preferential dissolution at their tips as discussed above. Alternatively, if the stress intensity within the glass should exceed a critical limit, they are likely to propagate catastrophically in stress corrosion cracks. In regions subjected to tensile stress, Si-O bonds are stretched and preferentially attacked by polar water molecules which become inserted between the silicon and oxygen atoms in the Si-O-Si glass network. In consequence, Si-O bonds are broken and replaced by Si-O-H/H-O-Si groupings where the vertical bar represents a rupture. This causes a crack to propagate inwards^{8,10}. The propagation distance is primarily controlled by the stress intensity at the tip of the crack. With an isolated stress centre, the stress intensity decreases with distance and crack growth is ultimately halted. If, however, the entire specimen is also subjected to an overall tensile or shear stress exceeding the critical limit, the crack will propagate through the entire specimen. Alternatively, if there is a high spatial density of stress centres (see below), their localized crack propagation envelopes may intersect, with catastrophic consequences.

After burial in geological repositories, nuclear waste glasses are likely to be subject to additional critical stresses arising from the effects of radiation damage on included crystalline phases. Borosilicate glasses produced on a large scale for HLW immobilization frequently contain a few per cent of dispersed crystalline phases, including metallic alloys, a fluorite-type phase $Ce^{4+}REE^{3+}O_{2-x}$ spinels, silicates and molybdates^{1,2} (where REE is the rare-earth element). The volume expansions exhibited by some of these crystalline phases after strong irradiation from α particles may be as high as several per cent, whereas glass does not usually expand beyond 1% (ref. 1). Localized stresses will be induced in the glass surrounding the damaged crystals. These stresses may greatly exceed the thermally-induced stresses around the spinel crystals in the present glass, which are caused by differential volume changes <1%.

In these circumstances, it seems that localized stresses around many of the larger (for example, $\sim 50\ \mu\text{m}$) crystalline phases could eventually exceed the critical levels required for the initiation of stress corrosion cracking on surfaces, which are in contact with groundwater, or even with atmospheric water vapour. The capacity of nuclear waste glasses to withstand

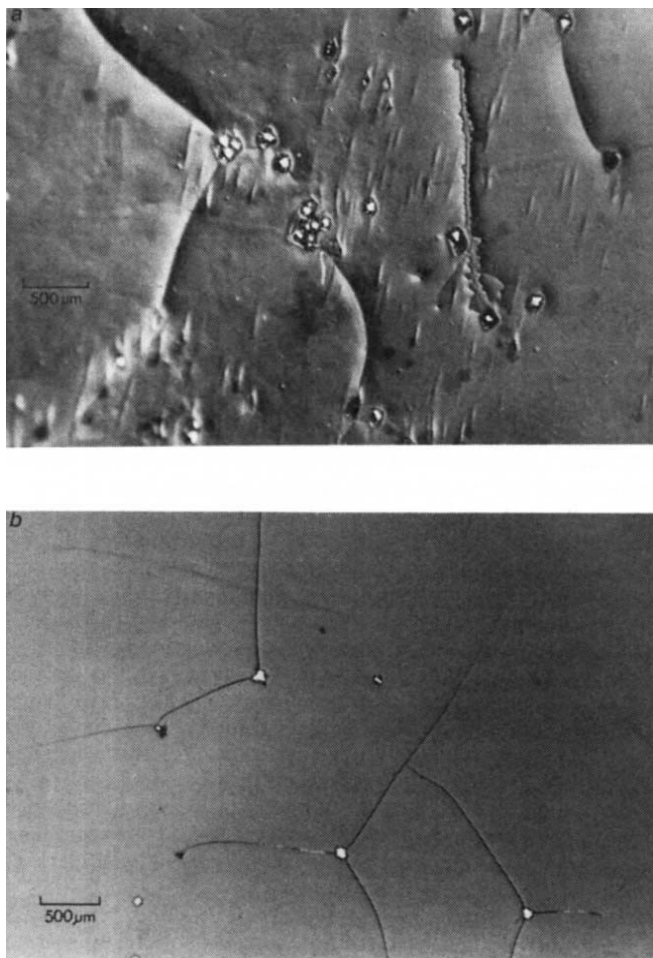


Fig. 3 Originally planar polished surfaces of glass sample from the interior of specimen after leaching. In a, spinel crystals now sit in deep pits formed by selective leaching of stressed envelopes. Note development of grooves associated with spinel crystals. These are believed to represent incipient stress corrosion cracking.

ultimate disintegration because of these factors is open to question.

The considerations advanced above suggest that susceptibility to stress corrosion cracking should be an important criterion in the evaluation and selection of wasteforms for high level nuclear waste.

We thank Dr B. Lawn of the US Bureau of Standards for discussion and constructive criticism. The investigation was partially supported by a grant provided by the National Energy Development and Demonstration Program of the Commonwealth Department of Resources and Energy.

Received 20 February; accepted 25 July 1984.

1. Turcotte, R. P. & Roberts, F. P. in *Ceramic and Glass Radioactive Waste Forms* (eds Readey, D. & Cooley, C.) 65-82 (Summary of workshop at ERDA-Germantown, CONF 770-102, 1977).
2. Turcotte, R. P., Wald, J. W. & May, R. P. in *Scientific Basis for Nuclear Waste Management* Vol. 2 (ed. Northrup, C.) 141-146 (Plenum, New York, 1980).
3. Ross, W. A. in *Ceramic and Glass Radioactive Waste Forms* (eds Readey, D. & Cooley, C.) 49-64 (Summary of workshop at ERDA-Germantown, CONF 770-102, 1977).
4. Laude, F., Vernaz, E. & Saint-Gauders, M. in *Scientific Basis for Nuclear Waste Management* Vol. 5, (ed. Lutze, W.) 239-247 (North-Holland, Amsterdam, 1982).
5. Wiederhorn, S. M. in *Ceramic and Glass Radioactive Waste Forms* (eds Readey, D. & Cooley, C.) 215-234 (Summary of workshop at ERDA-Germantown, CONF 770-102, 1977).
6. Wiederhorn, S. M. & Bolz, L. H. *J. Am. ceram. Soc.* **53**, 543 (1970).
7. Wiederhorn, S. M., Fuller, E. & Thomson, R. *Met. Sci.* **14**, 450 (1980).
8. Michalske, T. & Freeman, S. *Nature* **295**, 511 (1982).
9. Swain, M., Lawn, B. R. & Burns, S. *J. Mat. Sci.* **9**, 175 (1974).
10. Lawn, B. R. *J. Am. ceram. Soc.* **66**, 83 (1982).

A novel ignition device for the internal combustion engine

A. J. J. Lee & F. J. Weinberg

Department of Chemical Engineering and Chemical Technology,
Imperial College of Science and Technology, London SW7 2BY, UK

Although the recent rapid proliferation of research into plasma jet ignition¹⁻¹⁶ has partly been prompted by the requirements of continuous combustion and pollutant removal¹⁷⁻²⁵, the main motive has been to extend the lean operability limit of the internal combustion engine. This trend to leaner mixtures has been stimulated by the need to improve engine emission characteristics and efficiencies. Because optimum conditions occur near the lean misfire limit, a plug providing increased rates of flame propagation and more effective and reliable ignition has been sought; pulsed plasma jets offer much promise but use too much power. In these devices, the igniting jet is generated by explosive gas expulsion through an orifice, engendered by a short-duration arc discharge to which the plasma medium is exposed while confined by the walls of a cavity within the plug. Such plasma jets, and particularly those fed with specific plasma media, have proved capable of extending the flammable fuel-air mixture range both at the fuel lean and at the rich limit, and increase the speed at which flames propagate following ignition. This is thought to be due to the generation of turbulence and free radicals. We report here the development and testing of an ignition plug based on two sparks at the extremities of an internal cavity. This design combines much of the effectiveness of a plasma jet with the small power consumption, and consequently low rates of wear, of a conventional sparking plug.

From the point of view of practical applicability, plasma jets suffer from a major shortcoming; they use appreciably larger amounts of energy than do conventional automobile ignition systems. This results not only in increased consumption of electrical power but also in much greater rates of electrode wear. The former may be improved by redesigning the electrical supply system for greater efficiency, as the small proportion of the total energy which eventually reaches the jet¹⁰ is not inordinately larger than conventional spark energies. The latter, however, restricts plasma plug life to, at best, tens of hours. Thus we set out to design and test an ignition plug²⁶ which would combine some of the essential features of plasma jet ignition with the energy requirements of a conventional spark plug.

The essential features seem to be the production of a high-velocity gas jet which contains at least some regions or pockets of high-temperature gas within which active species, such as free radicals, reside. The spark channel itself, being at an extremely high temperature, is a sufficient source of the latter—without requiring the dissipation of a lot of energy in its creation. The main energy requirement in plasma jets (apart from losses due to the large currents used) seems to be associated with the generation and expulsion of the jet. If this could be contrived by drawing on the large amount of chemical energy in the fuel rather than on the electrical input, the major need for large amounts of electrical power would be removed. The plug design²⁶ in Fig. 1 achieves this aim as follows. Of the two sparks in series, the one at the base of the cavity is intended to ignite the flammable mixture compressed into the cavity by the advancing piston. The spark channel across the mouth of the cavity then rides on top of the ejected column of gas. For very lean mixtures, or fuels that are particularly difficult to ignite, small amounts of fuel or other substances which will promote reaction within the cavity may be introduced. Simple methods for providing the cavity with minute flows of gas, wetting it with small amounts of liquid fuel or furnishing it with solid hydride inserts have been developed in research on plasma jets^{8,9,11,13}.

There is evidence of jet formation and some gas expulsion through the orifice even in the absence of a flammable mixture

Table 1 Extension of incendiarity limits, both spark gaps broken down

Length (mm)	Cavity parameters Volume (mm ³)	Contents	% Limit extension	
			Lower	Upper
10	31.4	Test mixture	9.5	2.7
10	31.4	Oxygen mixture	14	56

Table 2 Increase of flame speed

Length (mm)	Cavity parameters Volume (mm ³)	Contents	% Improvement	
			Top spark only	Both sparks
1	3.14	Test mixture	47	64
2	6.28	Test mixture	48	59
4	12.6	Test mixture	42	63
7	22.0	Test mixture	55	74
10	31.4	Test mixture	45	80
1	3.14	Oxygen mixture	137	162
2	6.28	Oxygen mixture	132	200
4	12.6	Oxygen mixture	253	251
7	22.0	Oxygen mixture	282	270
10	31.4	Oxygen mixture	183	351

in the cavity. With a small cavity, even the small amount of energy that the internal spark imparts to its contents is sufficient to raise the temperature significantly, at least at atmospheric pressures. Thus if the spark energy were uniformly distributed across the 33 mm³ of the cavity, a doubling in the absolute room temperature would require some 10 mJ. Of course, the energy release from a stoichiometric mixture is almost an order of magnitude higher, even at atmospheric pressure, whilst, at engine pressures, the electrical contribution to the ejection of the jet would be expected to be relatively insignificant.

Practical tests to help elucidate the mechanism included a study of what happens when the two electrode gaps are shorted out in turn. Ignition at the base of the flammable contents of the cavity first ejects reactants into the gas to be ignited and eventually flame, if that has not been quenched within the cavity. Discharge at the upper electrode gap alone corresponds to conventional spark ignition only if the cavity is not filled with a flammable mixture. If flame propagation into the cavity can occur then the spark channel finds itself in a jet of hot products—again unless the flame is quenched. Comparing the incendiarity of the device in these various conditions with that of the two-discharge configuration gives much information on quenching and the mechanism by which the igniter functions generally. The quenching criterion is less restrictive in an engine than during tests at atmospheric pressure because quenching distance is inversely proportional to pressure. Quenching distance also decreases with increasing burning velocity and is about 10 times less for a stoichiometric mixture than at the limits of flammability. For this reason a series of experimental tests at atmospheric pressure were conducted with more vigorous flammable mixtures in the cavity to minimize quenching within it and simulate, in this respect, the most extreme engine conditions.

Before any fundamental work to optimize the plug design could be carried out, an opportunity arose to test the ignition device in a Ricardo experimental engine. It spite of the unoptimized cavity geometry, very promising reductions in burn times were obtained. As expected, these were greatest for the initial stages of propagation (8–9% during the first 10% of burn time) but were not inappreciable even when averaged over most of the flame travel (5–6% for 90% burn time).

In view of the difficulties encountered during engine experiments, more academic studies were carried out at atmospheric pressure with an adjustable plug (see Fig. 1b). This included provision for the feed of specific media as well as for variable cavity lengths and hence volumes. The most sensitive

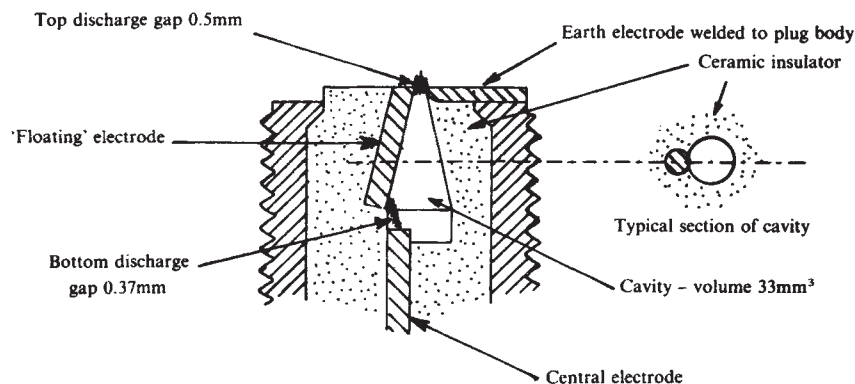
a

Fig. 1 Sections of tops of plugs used for: *a*, engine tests; *b*, laboratory experiments.

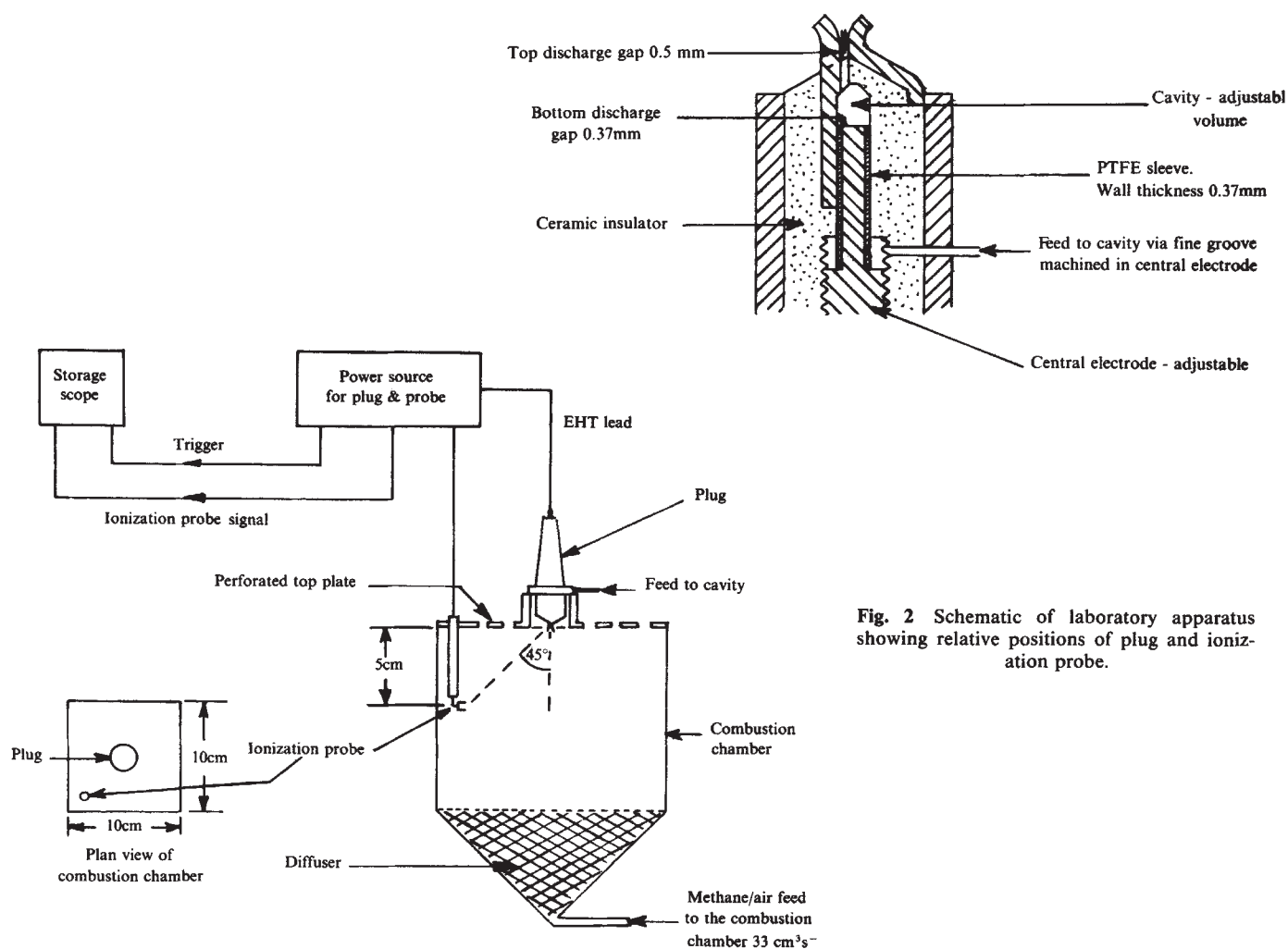
b

Fig. 2 Schematic of laboratory apparatus showing relative positions of plug and ionization probe.

measure^{9,10,16,21} of incendivity was found to be the stoichiometry of the leanest and richest mixtures which, when ignited at the centre of a vessel 10 cm square, would propagate flame all the way to the walls at least four times out of five. The other essential parameter is the speed of propagation of the flame. This was determined by measuring the time that the flame took to reach the ionization probe situated as shown in Fig. 2. The total stored energy available from the conventional ignition coil was of the order of 9 mJ.

The results for methane-air mixtures, based on several hundred firings, are listed in Tables 1 and 2. The 'percentage

improvement' refers to the decrease in minimum or increase in maximum fuel concentration which could be ignited, or to the increase in flame speed, all compared with the results for a conventional spark plug with a 2-mm gap. 'Test mixture' in the cavity signifies that the feed is the mixture under test in the vessel. 'Oxygen mixture' refers to the feeding into the plug of oxygen bubbled through mixtures of acetone and isopropanol in a saturator. The fastest burning mixtures studied produced stoichiometric conditions within the cavity. The flow in these experiments is minute, $\sim 0.083 \text{ cm}^3 \text{ s}^{-1}$, which is only sufficient to compensate for diffusion losses through the orifice and can

have no appreciable effect on the composition of the test mixtures¹⁶; this was stoichiometric methane-air for all the burning velocity measurements.

The lower and upper limit determinations were obtained using a plug without the extended electrodes shown in Fig. 1b, to avoid the quenching which these surfaces seem to induce at the lower limit. The spark duration was of the order of 1 ms. When it was reduced to 0.1 ms, no extension of limits of incandescence was observed, except for the oxygen mixtures, presumably because the spark at the mouth of the cavity expired before the jetting action became effective. Results with the bottom spark only are not listed in Table 2 because no ignition was achieved with the test mixture in the cavity, whilst ignition occurred only sporadically and irreproducibly with the oxygen mixture. When ignition did occur, however, burn times were virtually the same as for the two-spark system.

The new ignition device can extend the limits of incandescence and the rates of flame propagation—both sets of results compare favourably with the performance of high-energy (several joules stored) plasma jets (see refs 9, 16). The very large extension of the rich flammability limit for the more vigorously burning plasma media agrees with the plasma jet studies of ref. 16 and, by analogy, may be expected to be accompanied by large reductions of soot generation. The two-spark system consistently achieves large improvements over conventional sparking plugs. When the cavity is filled with the stoichiometric oxygen-fuel mixture, ignition by the top spark alone can produce very similar results—at least for cavities of intermediate lengths. The same is true for ignition by the bottom spark alone, though this is erratic and often does not occur at all. These results make good sense because, in the case of ignition with the top spark, propagation into the cavity results in the expulsion of a hot product jet immediately; in contradistinction to the spark at the base of the cavity, which will eject cold reactants until the flame reaches the top of the cavity—if it is not quenched before then. Unless a second spark is present at the top to ignite this cold reactant jet, the generation of turbulence (cold) is the most the jet can achieve. As the gas expansion due to combustion has been dissipated by the time that the flame reaches the top of the cavity, following ignition at the base alone, (all the jet velocities are readily calculable in terms of burning velocity and flame area) this type of 'torch ignition' would only occur when unquenched flame is ejected.

It is not possible to simulate cavity quenching in engine conditions accurately by feeding the plug any specific mixture, if only because a practical igniter has to cope with various throttle openings. The stoichiometric fuel-oxygen mixture used here was designed to represent one extreme, with burning velocities in excess of any that are likely to be obtained in an engine. It may be that it is only in such extreme conditions that either of the single spark systems generates a jet approaching the performance of the double spark configuration. The effectiveness of the plug in various conditions will also depend on the cavity diameter and orifice geometry, and further studies of the influence of these parameters are in progress. However, although single sparks at the top or the bottom of the cavity can sometimes produce considerable improvements over conventional sparking plugs, the double spark system produces large improvements consistently. It offers the advantages which have been claimed for plasma ignition without drawing any more electrical power than conventional spark plugs currently used in internal combustion engines.

We thank Mr. E. L. Marshall (BP Research Centre, Sunbury) for engine tests, Dr Ivan Vince who participated in some of the experimental work and the Wolfson Foundation for financial support to A.J.J.L.

Received 14 June; accepted 13 August 1984.

- Weinberg, F. J. *Inst. Mech. Eng. Symp. Combustion in Engineering*, Oxford, 65 (1983).
- Waterson, K. thesis, Oxford Univ. (1973).
- Topham, D. R., Smy, P. R. & Clements, R. M. *Combustion Flame* **25**, 187 (1975).
- Wyczalek, F. A., Frane, D. L., Neuman, J. C. *SAE pap.* 750349 (1975).

- Fitzgerald, D. J. *SAE Pap.* 76064 (1976).
- Asik, J. R., Piatkowski, P., Foucher, M. J. & Rado, W. G. *SAE Pap.* 770355 (1977).
- Dale, J. D., Smy, P. R. & Clements, R. M. *Combustion Flame* **31**, 173 (1978).
- Weinberg, F. J., Hom, K., Oppenheim, A. K. & Teichman, K. *Nature* **272**, 341 (1978).
- Orrin, J. E., Vince, I. M. & Weinberg, F. J. *18th Symp. int. on combustion* 1755 (The Combustion Institute, Pittsburgh (1981)).
- Carleton, F. B., Vince, I. M. & Weinberg, F. J. *19th Symp. int. on Combustion* 1523 (The Combustion Institute, Pittsburgh, 1982).
- Tozzi, L. & Dabora, E. K. *19th Symp. int. on Combustion*, 1467 (The Combustion Institute, Pittsburgh, 1982).
- Pitt, P. L. & Clements, R. M. *Combust. Sci. Technol.* **55**, 555 (1982).
- Grant, J. F., McIlwain, M. E. & Marram, E. P. *Combust. Sci. Technol.* **30**, 171 (1983).
- Clements, R. M., Smy, P. R. & Dale, J. D. *Combust. Flame* **42**, 287 (1981).
- Cetegen, B., Teichman, K. Y., Weinberg, F. J. & Oppenheim, A. K. *SAE Pap.* 80042 (1980).
- Vince, I. M., Vovelle, C. & Weinberg, F. J. *Combust. Flame* **105**, 56 (1984).
- Harrison, A. J. & Weinberg, F. J. *Proc. Soc. A321*, 95 (1971).
- Kimura, I. & Imajo, M. *16th Symp. int. on combustion*, 809 (The Combustion Institute, Pittsburgh, 1976).
- Kimura, I., Aoki, H. & Kato, M. *Combustion Flame* **42**, 297 (1981).
- Warris, A.-M. thesis, Univ. London (Imperial College) (1983).
- Warris, A.-M. & Weinberg, F. J. *20th Symp. int. on Combustion* (The Combustion Institute, Pittsburgh, 1984).
- Hilliard, J. C. & Weinberg, F. J. *Nature* **259**, 556 (1976).
- Behbahani, H. F., Fontijn, A., Muller-Dethlefs, K. & Weinberg, F. J. *Combust. Sci. Technol.* **27**, 123 (1982).
- Chan, A. K. F., Hilliard, J. C., Jones, A. R. & Weinberg, F. J. *J. Phys. D13*, 2309 (1980).
- Behbahani, H. F., Warris, A.-M. & Weinberg, F. J. *Combust. Sci. Technol.* **30**, 289 (1983).
- British Patent Application No. 827009, PCT/GB/83/00253 (October 1982).

Climatic impact of explosive volcanic eruptions

P. M. Kelly & C. B. Sear

Climatic Research Unit, School of Environmental Sciences,
University of East Anglia, Norwich NR4 7TJ, UK

Major explosive volcanic eruptions inject ash and gas into the upper atmosphere, producing aerosol layers which can affect the global energy balance and climate¹. Empirical studies have shown that major eruptions can produce a decrease in surface air temperature of up to a few tenths of a degree Celsius over the Northern Hemisphere land masses and that the effects may last for 2 or 3 yr (refs 2–4). This temperature decrease has been simulated by numerical models using realistic estimates of the nature of the aerosol cloud¹. Previous empirical studies of volcanic effects have examined fluctuations in monthly, seasonal or annual climate data, but generally only at a frequency of one observation per year. This has rendered determination of the timing of the onset of effects during the first year impossible. Using continuous monthly surface air temperature for the Northern Hemisphere land masses, we resolve the month-by-month development and decay of the initial climatic impact. In the case of Northern Hemisphere eruptions, abrupt cooling occurs during the first two to three months, which is more rapid than previously assumed.

Dates of major eruptions during the study period, 1881–1980, were extracted from records of volcanic activity based on geological evidence^{5,6}, on anecdotal, geological and other evidence^{7–10} and on conductivity (acidity) profiles from ice cores¹¹. Nine events were selected for study (Table 1). The selection criteria are summarized in the notes accompanying Table 1. As this study is concerned primarily with the initial climatic impact, the volcanic events were stratified into Northern Hemisphere and Southern Hemisphere samples. During the first few months of its lifetime, a volcanic cloud will be restricted to the hemisphere of injection, even in the case of low-latitude volcanoes¹². Two superposed epoch analyses were undertaken to define the Northern Hemisphere temperature response to the Northern Hemisphere and Southern Hemisphere eruptions. We used a recent compilation of monthly surface air temperatures¹³ interpolated onto a regular grid from station observations before averaging over the Northern Hemisphere.

Superposed epoch analysis is commonly used in studies of the effects of volcanic activity on climate^{2–4}. Taking the date of each eruption event as month zero, temperature data for succeeding months were averaged to produce the composite volcanic signals seen in Fig. 1. The value for month zero in the

Table 1 Volcanic events selected for study

Date (Month, year)	Event	Lat.	Long.	VEI†	DVI†	Acidity†
Northern Hemisphere						
5, 1902	Pelé, Montagne (West Indies)	14.8° N	61.2° W	2 × 4	100	
5, 1902	Soufrière of St Vincent (West Indies)	13.4° N	61.2° W	4	300	
*10, 1902	Santa Maria (Guatemala)	14.8° N	91.6° W	6	600	
3, 1907	Ksudach (Kamchatka)	51.8° N	157.5° E	5	500	
6, 1912	Novarupta (Alaska Peninsula)	58.3° N	155.2° W	6	500	30
3, 1956	Bezymianni (Kamchatka)	57.1° N	160.7° E	5	30	
Southern Hemisphere						
8, 1883	Krakatau (Indonesia)	6.1° S	105.4° E	6	1000	55
6, 1886	Tarawera (New Zealand)	38.2° S	176.5° E	5	800	
4, 1932	Azul (Chile)	35.7° S	70.8° W	5	70	
3, 1963	Agung (Indonesia)	8.3° S	115.5° E	4	800	20

Several factors determine the climatic significance of a volcanic eruption. The explosivity of an eruption affects the height of injection and, in particular, whether or not the dust and gas enters the stratosphere where residence times are much longer than in the lower atmosphere. The dust ejected (which is largely silicate ash) affects the energy balance in the initial stages of the development of the volcanic cloud. The amount of sulphur is, however, considered to be a more critical measure of climatic significance¹. The initial injection of sulphur gases is rapidly transformed into sulphate aerosols (secondary aerosol production) which have a greater effect on the energy balance and a longer residence time than the particulate injection.

All volcanic histories are prone to error because of the rudimentary nature of the historical data available and can only provide a crude classification of eruption size and climatic significance⁹. An objective synthesis of the available information has, therefore, been used to identify the events most likely to be of climatic importance. Well-dated events with a VEI of ≥ 5 and/or a DVI of ≥ 300 were selected.

* Not included in the superposed epoch analysis as effects are likely to be obscured by eruptions earlier in 1902.

† VEI, Volcanic explosivity index^{5,6}; DVI, dust veil index^{7,8,10}, not weighted by extent of veil; acidity, global fallout (10^6 tons)¹¹. Acidity estimates are not available for many of the eruptions.

Table 2 Monthly standard deviations (°C) of the Northern Hemisphere average surface air temperature data (1881–1980)

January	February	March	April	May	June
0.64	0.54	0.42	0.35	0.31	0.28
July	August	September	October	November	December
0.23	0.25	0.27	0.39	0.44	0.50

Northern Hemisphere composite shown in Fig. 1 corresponds to the average over the eruption months listed in Table 1; the value for month 1 is then the average of the temperature data for June 1902, April 1907, July 1912 and April 1956, and so on. The superposition, or compositing, clarifies the volcanic signal by averaging out features not common to the individual events. These are, presumably, caused by non-volcanic factors and can be considered to be noise.

To avoid biasing the results towards the winter months when the variability is greatest (Table 2), the data were standardized before compositing. Each monthly temperature value was divided by the appropriate long-term (100-yr) standard deviation after subtraction of the long-term monthly mean. We have repeated the analysis using unstandardized data and the general

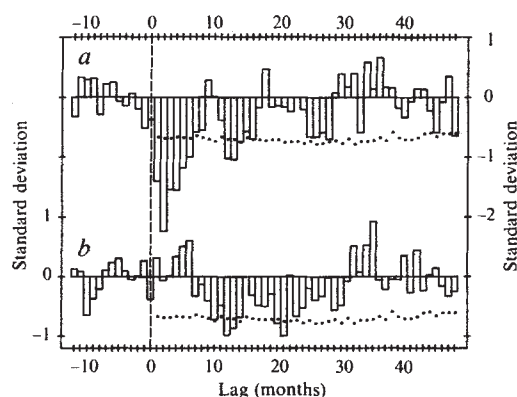


Fig. 1 Superposed epoch analyses of standardized monthly Northern Hemisphere surface air temperatures for the Northern Hemisphere eruption sample (a) and the Southern Hemisphere eruption sample (b). The data are expressed as deviations from prevailing temperature levels defined by the 12-month average for the period up to but not including the eruption date (month zero). To convert standard deviation units to °C, use Table 2. The near-horizontal line of dots indicates the one-tailed 5% significance level based on a Monte Carlo simulation. It should be noted that these data are largely representative of conditions over the land masses of the hemisphere as few data are available for ocean areas at present. Most of the eruptions studied took place during the early decades of the record for which the spatial coverage of the data is best over middle to high latitudes.

conclusions are not affected. To reveal the short-term response and avoid the effects of longer-term climatic changes, the data were then expressed as departures from the prevailing temperature level defined by the 12-month average for the period up to the eruption date (that is, month -12 to month -1). Use of longer averaging periods, of up to 5 yr, made little difference to the results.

Statistical significance was tested using a Monte Carlo technique¹⁴, so implicit account was taken of the particular statistical characteristics of the data under analysis (for example, systematic fluctuations caused by non-volcanic factors¹⁵). Values for months 1–48 were calculated as above using 1,000 sets of four randomly selected 'eruption' dates. The distribution of the resulting samples of 1,000 values was used to determine 5% significance levels separately for each of the 48 months. A one-tailed significance test was used, as it is generally accepted that surface cooling is the probable climatic effect¹.

The composite temperature departure sequences for the two samples are shown in Fig. 1. The Northern Hemisphere composite shows a marked response: a rapid decrease in temperature following month zero, with maximum cooling in month 2, then a slow recovery over the next couple of years. Temperatures return to the pre-eruption level during the third year. The maximum depression is about 2.2 s.d. corresponding to a cooling of ~ 1.4 °C in January and 0.5 °C in July. Individual departures during the first few months are clearly significant at the 5% level. The cumulative probability of the occurrence of a sequence of departures significant at the 5% level is, of course, extremely low. Statistically significant temperature depressions occur in all but six of the first 16 months and only three positive values occur during the first 28 months. The average depression over the first 12 months is between 0.3 and 0.4 °C depending on the month of the eruption. This agrees well with the results of previous analyses^{1–4}. The duration of statistically significant effects is around 18 months, although cooling is still apparent through to the early stages of the third year.

The Southern Hemisphere composite exhibits a similar, but delayed, signal. As we are now considering the effects of Southern Hemisphere eruptions on Northern Hemisphere climate, the delay is to be expected. Persistent cooling is first apparent about month 7 and is strongest during year 2. Depend-

ing on the time of year, the maximum cooling in any one month ranges between 0.2 and 0.6 °C and the average cooling during year 2 is ~0.2 °C, rather less than is often cited in the cases of Krakatau and Agung. The termination of appreciable effects at the beginning of the third year is similar to that seen in the Northern Hemisphere composite.

The similarity of the signal revealed by the two independent samples, allowing for the delay in the case of Southern Hemisphere events, strongly supports the reality of the volcanic effect. Nevertheless, there is a need for further verification by repeating the analysis for earlier eruptions and on a Southern Hemisphere temperature average. At present, however, suitable data are not available.

The composite volcanic signals seen in Fig. 1 are statistical generalizations and their applicability to individual events is not necessarily straightforward because of the background climate variability or noise. Temperature departures following the individual volcanic events are shown in Figs 2 and 3. With the composites from Fig. 1 in mind, the outline of the volcanic signal can be seen in most of these sequences. The notable exception is the case of Tarawera. Interestingly, the magnitude of the maximum response, in terms of standard deviation units, is similar in all the individual sequences (except that of Tarawera) although the character and size of the associated stratospheric injections differed. The apparent duration of the climate response does, however, vary from event to event, although the point where the volcanic signal becomes indistinguishable from the background noise is difficult to determine.

The most striking feature of our results is the speed of the climate system's response. Contrary to general belief, the maximum impact occurs when the volcanic cloud is far from being hemispherically distributed. The time scale of the development of the aerosol cloud produced by the eruptions of El Chichón, Mexico, in April 1982¹⁶ is relevant here. Satellite observations¹² of the stratospheric injection have shown that the aerosol cloud at 27 km reached maximum density in late May 1982 as particle growth rates were first balanced and then exceeded by sedimentation losses. The total mass of sulphate above 20 km reached a maximum about 15 weeks after the eruption¹⁷. Modelling of the chemistry involved suggests a similar 2-5-month gas-to-particle transformation time with maximum optical depths in lower northern latitudes occurring during summer 1982¹⁸. By the beginning of June 1982, the El Chichón aerosol cloud was homogeneous and circumglobal in longitudinal extent but limited in latitudinal span¹². Turco *et al.*¹⁹, modelling the net effect on hemispherically averaged vertical optical depth, find maximum departures occurring 1-3 months after the eruption, in agreement with our results. (Unfortunately, the exceptional warmth of 1981¹³ and the onset of cooling in early 1982, before the El Chichón eruptions, renders identification of the actual surface air temperature response difficult²⁰.)

Nevertheless, it is surprising that surface temperatures, even over land, should respond so quickly to the volcanic forcing with little, if any, delay produced by the thermal inertia of the climate system. The mechanism whereby a localized disturbance in the energy balance can produce a swift response in surface air temperature of hemispheric significance (although not necessarily hemispheric extent) warrants further investigation. As Ellsaesser has noted¹⁵, empirical studies concerning volcanic effects on climate tend to raise as many, if not more, questions than they answer. This is symptomatic of many studies of the causes of climatic change and is an almost inevitable byproduct of limited data availability and small sample size.

The strength of the immediate temperature response to volcanic injections revealed by our analysis may result in a limited predictive ability, and the accurate determination of the volcanic signal is relevant to the prompt detection of the climatic effects of increasing levels of atmospheric carbon dioxide²¹. We believe, however, that the major significance of this work lies in the fact that it challenges commonly held beliefs concerning the response time and the sensitivity of the climate system to external forcing.

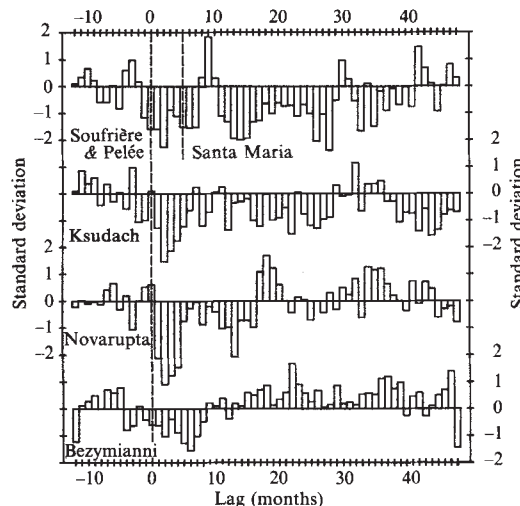


Fig. 2 Individual sequences of standardized monthly Northern Hemisphere temperatures following the eruption dates in the Northern Hemisphere sample (see Table 1). The data are expressed as deviations from prevailing temperature levels defined by the 12-month average for the period up to but not including the eruption date (month zero). To convert standard deviation units to °C, use Table 2.

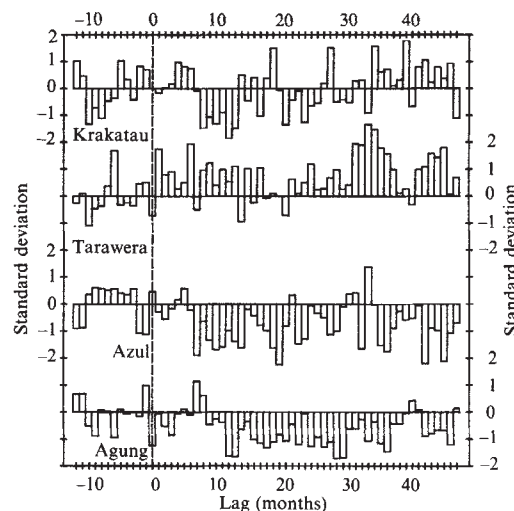


Fig. 3 Individual sequences of standardized monthly Northern Hemisphere temperatures following the eruption dates in the Southern Hemisphere sample (see Table 1). The data are expressed as deviations from prevailing temperature levels defined by the 12-month average for the period up to but not including the eruption date (month zero). To convert standard deviation units to °C, use Table 2.

Our results provide empirical support for the short response time suggested by recent attempts to simulate the climatic effects of a nuclear exchange^{19,22}.

This research was supported by US Office of Naval Research grant N0014-77-G-0074 and US Department of Energy contracts DE-ACO2-81EV10739 and DE-ACO2-79EV10098. C.B.S. was in receipt of a NERC Research Studentship. We thank all who commented on this research, particularly A. Robock, S. H. Schneider and T. M. L. Wigley, and acknowledge the inspiration of H. H. Lamb. E. M. Kelly provided programming assistance and the analyses were performed on 16-bit microcomputers.

Received 11 May; accepted 20 August 1984.

1. Newell, R. E. & Deepak, A. *Mount St Helens Eruptions of 1980: Atmospheric Effects and Potential Climatic Impact* (NASA, Washington, DC, 1982).
2. Mass, C. & Schneider, S. H. *J. Atmos. Sci.* **34**, 1995-2008 (1977).

3. Taylor, B. L., Gal-Chen, T. & Schneider, S. H. *Jl. R. met. Soc.* **196**, 175-199 (1980).
4. Self, S., Rampino, M. R. & Barbera, J. J. *J. Volcanol. Geother. Res.* **11**, 41-60 (1981).
5. Simkin, T. *et al. Volcanoes of the World* (Hutchinson Ross, Stroudsburg, 1981).
6. Newhall, C. G. & Self, S. *J. geophys. Res.* **87**, 1231-1238 (1982).
7. Lamb, H. H. *Phil. Trans. R. Soc. A* **266**, 425-533 (1970).
8. Lamb, H. H. *Climate Monitor* **6**, 57-67 (1977).
9. Kelly, P. M. & Sear, C. B. in *Atmospheric Effects and Potential Climatic Impact of the 1980 Eruptions of Mount St Helens* (ed. Deepak, A.) 293-298 (NASA, Conf. Publ. no. 2240, Washington DC, 1982).
10. Lamb, H. H. *Climate Monitor* **12**, 79-80 (1983).
11. Hammer, C. U., Hausen, H. B. & Dansgaard, W. *Nature* **288**, 230-235 (1980).
12. Barth, C. A. *et al. Geophys. Res. Lett.* **10**, 993-996 (1983).
13. Jones, P. D., Wigley, T. M. L. & Kelly, P. M. *Mon. Weath. Rev.* **110**, 59-70 (1982).
14. Haurwitz, M. W. & Brier, G. W. *Mon. Weath. Rev.* **109**, 2074-2079 (1981).
15. Ellsaesser, H. W. Preprint UCRL-89161, (Lawrence Livermore National Laboratory, Univ. California, Livermore, 1983).
16. Robock, A. *Nature* **301**, 373-374 (1983).
17. Thomas, G. E., Jakosky, B. M., West, R. A. & Sanders, R. W. *Geophys. Res. Lett.* **10**, 997-1000, (1983).
18. Capone, L. A. *et al. Geophys. Res. Lett.* **10**, 1053-1056 (1983).
19. Turco, R. P., Toon, O. B., Ackerman, T. P., Pollack, J. B. & Sagan, C. *Science* **222**, 1283-1292 (1983).
20. Sear, C. B. & Kelly, P. M. *Climate Monitor* **11**, 134-139 (1982).
21. Klein, W. H. in *Carbon Dioxide Review: 1982* (ed. Clark, W. C.) 215-242 (Oxford University Press, 1982).
22. Covey, C., Schneider, S. H. & Thompson, S. L. *Nature* **308**, 21-25 (1984).

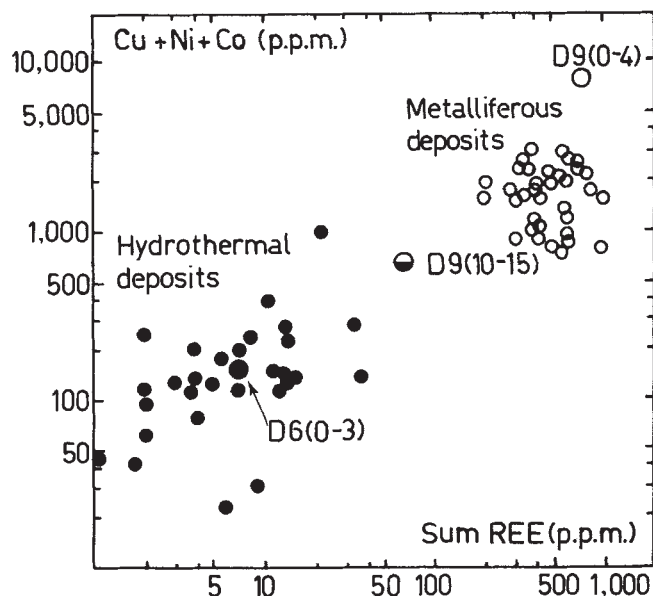


Fig. 1 Correlation between REE and Cu + Ni + Co concentrations in hydrothermal deposits and metalliferous sedimentary deposits. Data, characterized by smaller open and filled circles, are from ref. 10 corresponding to hydrothermal and metalliferous material from FAMOUS, Galapagos, Cyprus, Leg 54, Bauer deep, Marquesas zone, North Pacific^{4,19-22}.

Nd-Sr isotopic and REE constraints on the genesis of hydrothermal manganese crusts in the Galapagos

N. Clauer*, P. Stille*, C. Bonnot-Courtois† & W. S. Moore‡

* Centre de Sédimentologie et Géochimie de la Surface, Université L. Pasteur, 67084 Strasbourg-Cedex, France

† Equipe de Recherche Associée 765, Université Paris Sud, Orsay, France

‡ Department of Geology, University of South Carolina, Columbia, South Carolina 29208, USA

Manganese oxide crusts from the Galapagos spreading centre, interpreted to be of hydrothermal origin, have Nd isotopic compositions and rare-earth element (REE) concentrations that approach seawater values as the age increases. Numerous authors^{1,2} have cited hydrothermal processes along the mid-oceanic ridges to explain the composition of 'metalliferous' sediments. Other authors believe that these sediments, whether now located on a ridge axis or in some deep, were derived from hydrothermal activity near ridges and were modified during removal from these ridges³⁻⁶. Nd and Sr isotopic compositions as well as concentrations of REE and Cu, Ni and Co in hydrothermal Mn crusts of different ages from the Galapagos spreading area were determined to provide new information regarding the problem of the alteration behaviour with time of oceanic sediments. Our data suggest that hydrothermal crusts react with seawater and ultimately acquire the trace-element characteristics of hydrogeneous crusts.

During a 1972 oceanographic cruise to the Galapagos spreading axis⁷, dredging in the axial valley recovered a 2-4 cm thick Mn crust (station D6) consisting of a surface grey-banded zone covering a dark, friable zone. The latter material is todorokite, whereas the former consists of a mixture of todorokite and birnesite. The basement age is 0.3 Myr as estimated from magnetic anomalies.

At a second station (D9) located on a ridge about 25 km north-east of D6, fragments of another manganese oxide crust were also recovered on a 2.4 Myr old basement. This crust consists of a dark, iron-stained, 2-4-mm thick surface zone covering 4-6 cm of massive, grey material. The outer layer is amorphous material, and the underlying 4-6 cm is a mixture of todorokite and birnesite. Samples analysed are identified in Table 1.

Previous studies⁷ showed that the outer layer of the D9 crust was hydrogeneous, but that the 0.3-Myr old D6 and the 2.4-Myr old D9 crusts are of hydrothermal origin. This conclusion is

supported by the high accumulation rates measured by ²³⁰Th/²³⁴U dating of the Mn crusts, as well as by the young basement age determined by magnetic anomalies. Thus the growth rate for the D6 crust is of the order of 1-2 mm kyr⁻¹, 1,000 times faster than the growth rate of hydrogeneous Mn deposits.

A comparison of Cu + Ni + Co concentrations with REE concentrations (Fig. 1), provides a means of differentiating and characterizing hydrothermal and hydrogeneous Mn deposits. Hydrothermal deposits have REE and Cu, Ni and Co concentrations that are distinctly lower than those of hydrogeneous deposits. The data for the hydrothermal section of D9 fall in a region of the diagram that is intermediate between those of hydrogeneous deposits, here typified by its own outer coating, and those of hydrothermal deposits typified by the younger D6 crust.

In Fig. 2, the shale-normalized distribution of REEs⁸ in the hydrothermally-formed D6 crust is quite similar to those of other hydrothermal deposits from the Galapagos⁹ and from the FAMOUS area¹⁰. The crust shows a weak negative Ce anomaly and a slight increase in the heavy rare-earth elements (HREEs). The REE patterns of hydrothermally-formed parts of the D9 crust are significantly different from those of D6. Especially striking is the strong Ce anomaly and the marked similarity of the REE patterns to that of Pacific Ocean water and of authigenic marine sediments. The outer hydrogeneous layer of the same crust contains very high REE concentrations of about 765 p.p.m.; again the pattern shows a slight enrichment in the HREEs, but the negative Ce anomaly is less developed than in the hydrothermal portion.

The Sr isotopic ratios measured on 12 samples of the D6 and D9 crusts are remarkably constant. The average value is 0.70906 ± 4 (2σ mean) and thus agrees very well with present seawater Sr isotopic composition¹¹. The Sr isotopic ratio of seawater measured in the hydrogeneous outer coating of D9 was as expected. For the hydrothermally-formed parts of the crust, however, we suggest either that the Sr originally had seawater-like ratios or more probably that exchanges with seawater occurred after the crust was formed.

Albarede *et al.*¹² measured the Sr isotopic compositions of hydrothermal waters and deposits from the East Pacific Rise

Table 1 Sr-Nd isotopic compositions* and Cu, Ni, Co and REE concentrations in the Mn crusts from the Galapagos spreading centre

Depth (mm)	Sr (p.p.m.)	⁸⁷ Sr/ ⁸⁶ Sr†	¹⁴³ Nd/ ¹⁴⁴ Nd‡	Cu (p.p.m.)§	Ni (p.p.m.)§	Co (p.p.m.)§	Cu + Ni + Co (p.p.m.)			
Dredge D6										
0-3	969	0.70911 ± 18	—	43	100	<23	<166			
15-20	518	0.70917 ± 13	0.512609 ± 31	16	—	35	>51			
30-36	364	0.70915 ± 8	—	—	—	—	—			
Dredge D9										
0-4	1,457	0.70915 ± 5	0.512518 ± 48 0.512520 ± 40	936	6,300	909	8,145			
0-4	3,213	0.70922 ± 22	—	—	—	—	—			
0-4¶	642	0.70909 ± 11	—	—	—	—	—			
10-15	599	0.70913 ± 12	—	65	535	<50	<650			
23-30	1,502	0.70894 ± 13	—	—	—	—	—			
23-30¶¶	262	0.70918 ± 9	—	—	—	—	—			
51-58	757	0.70914 ± 10	0.512499 ± 50 #	—	—	—	—			
51-58	2,327	0.70911 ± 11	—	—	—	—	—			
51-58¶¶	278	0.70918 ± 7	—	—	—	—	—			
	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu	Total	La/Yb
Dredge D6										
0-3	2.55	2.74	1.5	0.33	0.12	0.09	0.52	0.10	8	4.9
30-36	2.96	4.07	2.1	0.42	0.15	0.12	0.39	0.07	10	7.6
Dredge D9										
0-4	219	281	178	37.3	9.40	6.80	27.9	5.40	765	7.8
10-15	23.3	8.17	19.5	3.83	1.25	1.00	5.82	1.13	64	4.0
51-58	29.7	7.60	32.5	6.40	1.98	1.60	5.46	0.98	86	5.4

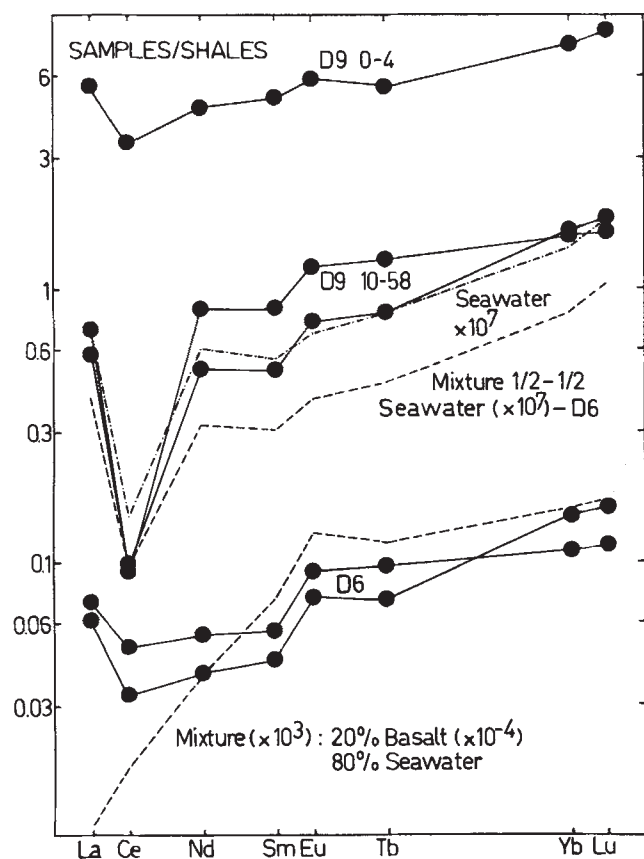
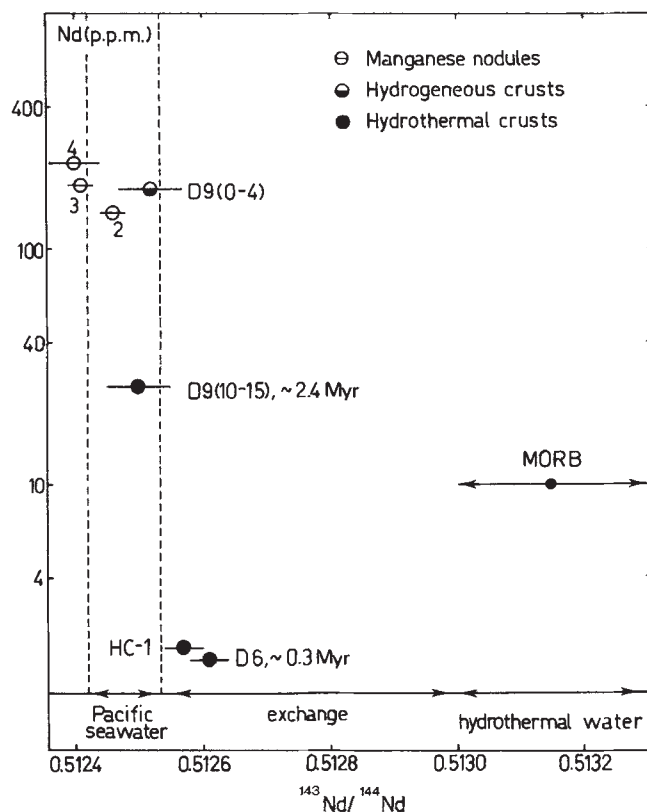
* Uncertainties are 2σ mean.† Ratios adjusted to 0.71022 mean value for the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the NBS 987 standard.‡ Normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$. $^{143}\text{Nd}/^{144}\text{Nd} = 0.51261 \pm 30$ for International Rock Standard BCR-1.

§ Values from ref. 16.

|| Values of the solutions after weak leaching in 1M HCl.

¶ Values of the residues after weak leaching in 1M HCl.

D9(10-58), no leaching.

**Fig. 2** Distribution patterns of the REE in the Mn-crusts from the Galapagos spreading centre. Seawater $\times 10^7$.**Fig. 3** $^{143}\text{Nd}/^{144}\text{Nd}$ plotted against Nd-concentrations. Hydrogeneous nodules 2-4 and hydrothermal crust HC-1 are from ref. 23. Nd isotopic composition values of Pacific seawater are from ref. 24.

(EPR) and showed that the hydrothermal system may be basically handled as a two component system in which endmembers are (1) the ambient seawater, and (2) a hot hydrothermal solution with $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.703, close to, but significantly higher than, the Sr isotopic ratio of the surrounding fresh basalts. Similar observations were made by Vidal and Clauer¹³ in their combined Pb–Sr isotopic study of basalts and sulphides from the EPR. They found that the Sr isotopic compositions of the hydrothermally-formed sulphides were intermediate between those of basalt and seawater.

Based on these studies and the strong evidence for the hydrothermal origin of the D6 and lower parts of the D9 crusts, we suggest that originally both seawater and hydrothermal Sr were incorporated in the Mn crusts. Later, after a long exposure to seawater, the original Sr isotopic compositions changed to present seawater values.

This suggestion is supported by the Nd isotopic compositions and Nd concentrations obtained on D6 and D9 crusts (Fig. 3). The isotopic composition of hydrothermal water is suggested to be mid-ocean ridge basalt (MORB)-like. The hydrogenous samples, including the outer coating of the D9 crust, are characterized by high Nd concentrations ranging between 150 and 300 p.p.m. and Nd isotopic compositions that are the same as Pacific ocean water and agree within error limits with those found by Goldstein and O'Nions¹⁴ on the same D9 crust. In addition, the hydrothermal portion of the D9 crust has a seawater Nd isotopic composition, but its Nd concentration is intermediate between the data for the remaining hydrothermal and hydrogenous Mn crusts. The two hydrothermal samples (HC-1 and D6) have very low Nd concentrations of <2 p.p.m. and Nd isotopic compositions that are higher than the upper limit for Pacific Ocean water. This observation indicates that the Nd in these deposits has a volcanogenic component.

Corliss¹⁵ demonstrated that hydrothermal leaching of submarine basalts could supply all excess REEs in the hydrothermal and hydrogenous crusts. His geochemical balance calculations further show that the REE concentrations in hydrothermal mineralizing solutions are significantly greater than the concentrations of the seawater reservoir. This is supported by new measurements on high temperature hydrothermal waters from the EPR by Michard *et al.*¹⁶ who demonstrated that REE concentrations in hydrothermal fluids are approximately 1,000 times those of seawater.

Assuming that leaching of basaltic material is the main mechanism which produces excess REE in the hydrothermal solutions and that its Nd isotopic composition and REE distribution was similar to MORB, then ~15–20% of the total Nd in the hydrothermal Mn crust of D6 must have been derived from a magmatic source and 80% from Pacific seawater.

Because of the extremely low REE concentrations in seawater, its mixing with a basalt source in the above-mentioned proportions will still produce a basaltic REE pattern. However, as shown in Fig. 2 only the HREEs in the hydrothermal crust of D6 reflect a basaltic component. The strong light rare-earth element (LREE) depletion, typical for MORB, is not observed. Thus, if hydrothermal leaching of basalts was the main mechanism which accumulates REEs in the hydrothermal solutions it did not fractionate the HREE but it enriched the LREE relative to the HREE. This suggestion is in good agreement with the geochemical mass balance calculations of Corliss¹⁵, which demonstrate that in hydrothermal leaching of basalts three times more Ce than Sm accumulates in the solutions. It also agrees with the statement of Michard *et al.*¹⁶ who observed a large enrichment of LREE in hydrothermal waters relative to seawater.

The 2-Myr older hydrothermal portion of the D9 crust was possibly formed in different conditions than that of the D6 crust. A larger seawater or even detrital component might be responsible for the significantly lower Nd isotopic compositions and the REE concentrations that are higher than usual for hydrothermal deposits.

The strong similarity in REE patterns to those of ocean water might indicate that seawater rather than detrital sediments was

responsible for modifying the chemical characteristics of these crusts with time. We, therefore, suggest that the REE patterns of D9 reflect mixing between a hydrothermal and a seawater pattern. The theoretical mixing REE pattern is shown in Fig. 2. If our suggestion is correct and the crust originally had a REE pattern similar to that of the 2 Myr younger D6 crust, then the material must have undergone extensive REE exchange with seawater, possibly by a diffusion process^{17,18}. Such post-depositional processes seem to alter significantly the composition of elements and to erase the original hydrothermal signature.

We thank J. Abrecht, P. J. Patchett and J. R. O'Neil for helpful discussions and English corrections. This work was funded by a grant from the Swiss National Science Foundation to P.S.

Received 23 March, accepted 20 July 1984.

1. Bostrom, K. & Peterson, M. N. *A. Mar. Geol.* **7**, 427–447 (1969).
2. Bostrom, K. *Earth planet. Sci. Lett.* **9**, 348–354 (1970).
3. Bischoff, J. L. & Sayles, F. L. *J. sedim. Petrol.* **42**, 711–724 (1972).
4. Dymond, J., Corliss, J. B., Heath, G. R., Field, C. W. & Dasch, E. J. *Bull. geol. Soc. Am.* **84**, 3355–3372 (1973).
5. Bischoff, J. L. & Rosenbauer, R. J. *Earth planet. Sci. Lett.* **33**, 379–388 (1977).
6. Edmond, J. M. *et al. Earth planet. Sci. Lett.* **46**, 19–30 (1979).
7. Moore, W. S. & Vogt, P. R. *Earth planet. Sci. Lett.* **29**, 349–356 (1976).
8. Hoffert, M. *Sci. Géol. Mém. Strus.* **61**, 231 (1980).
9. Haskin, M. A. & Haskin, L. A. *Science* **154**, 507–509 (1966).
10. Bonnot-Courtois, C. *Mar. Geol.* **39**, 1–14 (1981).
11. Faure, G. in *Numerical Dating in Stratigraphy* (ed. Odin, G. S.) (Wiley, New York, 1982).
12. Albareda, F., Michard, A., Minster, J. F. & Michard, G. *Earth planet. Sci. Lett.* **55**, 229–236 (1981).
13. Vidal, Ph. & Clauer, N. *Earth planet. Sci. Lett.* **55**, 237–246 (1981).
14. Goldstein, W. L. & O'Nions, R. K. *Nature* **292**, 324–326 (1981).
15. Corliss, J. B. *J. geophys. Res.* **76**, 8128–8138 (1971).
16. Michard, A., Albareda, F., Michard, G., Minster, J. F. & Charlou, J. L. *Terra cognita* **3**, 169 (1983).
17. Krishnaswami, S. & Cochran, J. K. *Earth planet. Sci. Lett.* **40**, 45–62 (1978).
18. Moore, W. S. *et al. Earth planet. Sci. Lett.* **52**, 151–171 (1981).
19. Dymond, J., Corliss, J. B. & Heath, G. R. *Geochim. Cosmochim. Acta* **41**, 741–753 (1977).
20. Corliss, J. B., Lyle, M., Dymond, J. & Crane, K. *Earth planet. Sci. Lett.* **40**, 12–24 (1978).
21. Courtois, C. & Desprairies, A. C. R. *Somm. Soc. Geol. Fr.* **5**, 242–245 (1978).
22. Courtois, C. & Hoffert, M. *Bull. Soc. géol. Fr.* **19**, 1245–1251 (1977).
23. Piepgras, D. J., Wasserburg, G. J. & Dasch, E. J. *Earth planet. Sci. Lett.* **45**, 223–236 (1979).
24. Piepgras, D. J. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **50**, 128–138 (1980).

Efficiency of petroleum expulsion from shale source rocks

D. Leythaeuser, M. Radke & R. G. Schaefer

Institute for Petroleum and Organic Geochemistry (ICH-5), KFA-Jülich, PO Box 1913, D-5170 Jülich, FRG

The limited data available on the mechanism and the efficiency of the processes by which petroleum is expelled in the subsurface from source rocks into adjacent reservoir rocks are mostly qualitative^{1,2}. We present here material balance-type comparisons of sample series extending from the centre portions of two adjacent hydrocarbon source rock units towards their outer edges³, which enable expulsion efficiencies to be determined. In certain parts of the shale the expulsion efficiencies for total extract and its saturated and aromatic hydrocarbon fractions, have much higher values than previously thought (reaching up to 80% for the total extract) which increase towards possible secondary migration avenues. The expulsion is associated with fractionation effects, such as those based on polarity differences. Also, expulsion efficiencies were found to be related to the degree of hydrocarbon saturation of the pore system: they are higher for a rich, oil-prone source rock unit compared with a poorer quality one, which had generated less hydrocarbons.

Using geochemical techniques described elsewhere^{4,5} 15 samples of conventional cores were analysed from a ~30-m thick organic matter-rich shale interval in the Kökelsumer Heide no. 1 well penetrating Upper Carboniferous coal-bearing strata in the northern Ruhr district, FRG. From the variation of its organic carbon content and kerogen composition⁶ (as defined by the hydrogen index HI, ref. 7), this shale could be subdivided

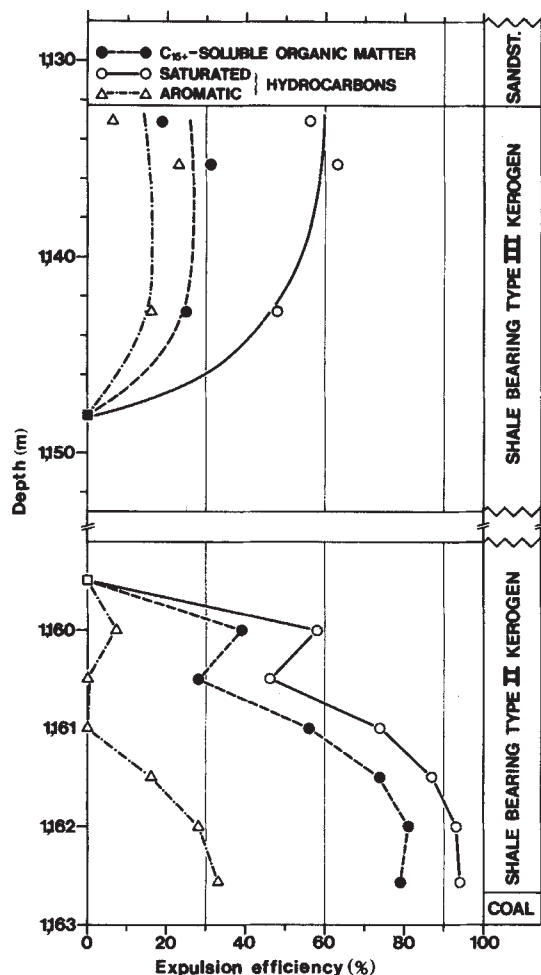


Fig. 1 Efficiency of expulsion of C_{15+} -soluble organic matter and saturated and aromatic hydrocarbons plotted against depth for the upper and lower depletion intervals of shale unit 1,132.5–1,162.6 m in the corehole Kökelsumer Heide No. 1, Ruhr district, FRG. The calculation of expulsion efficiencies^{10,11} was based on the assumption that samples 1,148.1 m and 1,159.5 m have the hydrocarbon concentrations which resemble most closely those generated *in situ* (unmodified) for the upper and lower shale interval respectively.

into two intervals (Fig. 1, right hand column):

- (1) From 1,132.5 to 1,158.0 m a medium grey shale with modest organic carbon content (mean, 0.8%) and bearing a hydrogen-lean type III kerogen⁸ (mean HI 61.2 mg hydrocarbons per g organic carbon) was penetrated.
- (2) From 1,158.0 to 1,162.6 m a black shale of high organic carbon content (mean, 3.9%) and bearing a hydrogen-rich, oil-prone type II kerogen⁸ (mean HI, 175 mg per g organic carbon) occurs. Stratigraphically, this interval is the 'Katharina shale', which was deposited during a short marine transgression and directly overlies the Katharina coal seam⁹.

Within each of these intervals the kerogen composition remains uniform except for the lowermost two samples of (2) where a high proportion of reworked and oxidized organic matter is thought to have been admixed with the type II kerogen⁶. Formation of petroleum hydrocarbons has advanced in both shales to beyond the peak generation stage as indicated by a maturity level of the organic matter equivalent to 1.1% mean vitrinite reflectance. The qualitative effects of hydrocarbon expulsion from the above-defined source rock intervals have been reported previously⁶. We now concentrate on quantifying some of these expulsion effects.

Samples from the upper and the lower edge of shale interval 1,132.5–1,162.6 m had systematic differences in concentration and composition of extractable hydrocarbons compared with samples from the centre⁶. Using an established concept^{10,11} we

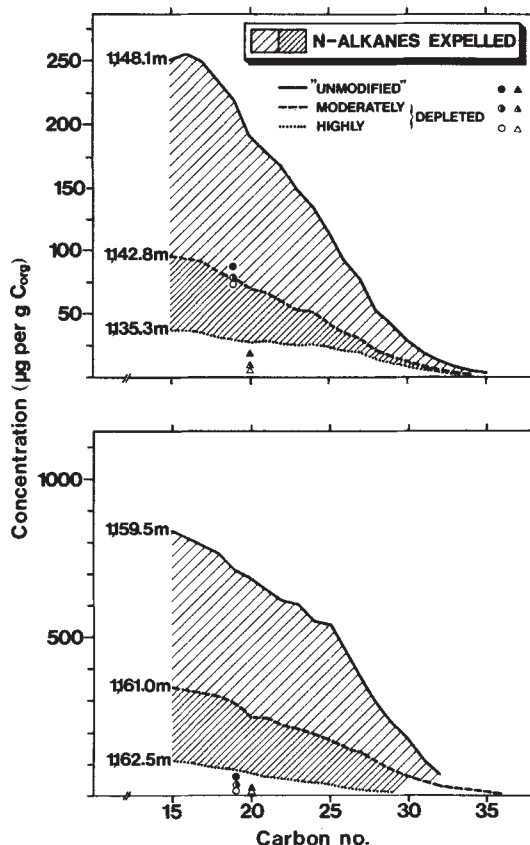


Fig. 2 Comparison of the absolute amounts of individual normal and isoprenoid alkanes ($\mu\text{g per g } C_{\text{org}}$) based on quantitative evaluation of the gas chromatograms for selected samples from the upper (1,148.1, 1,142.8 and 1,135.3 m) and lower depletion intervals (1,159.5, 1,161.0 and 1,162.5 m) of the shale unit shown in Fig. 1. The designation of samples as 'unmodified', 'moderately' and 'highly depleted' is explained in the text. Concentration differences between the three samples from each depletion zone represent hydrocarbon mixtures expelled from the indicated interval during primary migration. Circles, pristane; triangles, phytane.

now attempt to quantify these observations. For this purpose, the samples at 1,148.1 m and 1,159.5 m were assumed to have the hydrocarbon concentrations, which resemble most closely those generated *in situ* (that is, those within each shale unit relatively least affected by expulsion losses). They are referred to as the 'unmodified' samples, although they may also have lost some of their originally-generated hydrocarbons.

The percentage decrease in total extract and extractable hydrocarbons, shown in Fig. 1, for all samples closer to the upper and the lower shale contact respectively are interpreted as reflecting the expulsion efficiency. Over the uppermost 12-m and the lowermost 2.5-m intervals, the expulsion efficiencies for the total extract and its saturated and aromatic hydrocarbon fractions increase strongly towards the outer shale contacts: the edges of the shale have been preferentially depleted by expulsion. At any given depth level within both depleted intervals the expulsion efficiency is highest for the saturated hydrocarbons and lowest for the aromatic hydrocarbons; that of the total extracts is between the two. These trends are more regular in the lower depletion interval, where expulsion efficiencies increase within a narrow interval of only 2.5 m for the total extract to almost 90%, and for the saturated and aromatic hydrocarbons up to 94 and 33% respectively. The drainage avenue into which the expelled hydrocarbons were transported is obvious for the upper depletion zone (the overlying sandstone), while it is not as clear for the lower one. Several thin

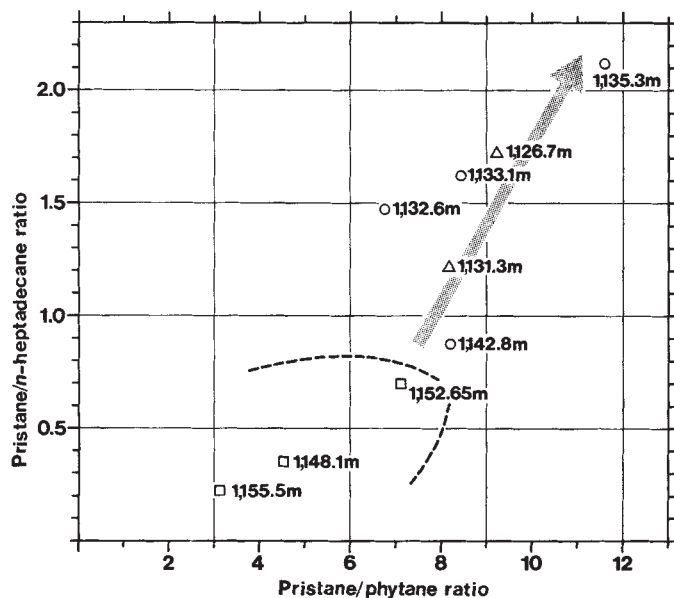


Fig. 3 Pristane/*n*-heptadecane plotted against pristane/phytane concentration ratios for all samples (identified by depth) from the type III kerogen-bearing source rock unit shown in Fig. 1. Note that the original composition largely preserved in the centre of this shale unit (squares) is altered more and more with increasing degree of hydrocarbon expulsion as indicated by the depleted samples (circles, from edge of this shale unit; triangles, thin, highly depleted shale interbeds from overlying sandstone unit). Arrow indicates the shift with increasing degree of expulsion.

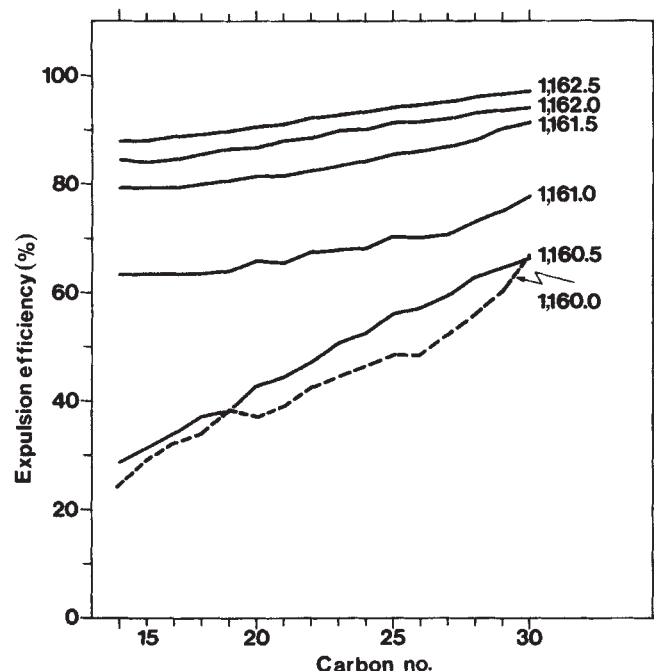


Fig. 4 Efficiencies of expulsion (as % of the unmodified sample 1,159.5 m) of individual *n*-alkanes as a function of carbon number and depth for all samples from the lower depletion interval (defined in Fig. 1), corehole Kökelsumer Heide No. 1, Ruhr district, FRG.

sandstone layers between 1,167.0 and 1,180.0 m are possible avenues because the Katharina coal seam shows a similarly severe depletion of hydrocarbons as previously observed nearby¹². That the expulsion efficiencies are highest at 1,135.3 m within the upper depletion interval, and not at the shale/sandstone contact itself, suggests that increased depletion into a fracture zone between 1,135.0 and 1,135.2 m has occurred⁶.

The conclusions reached from Fig. 1 support the hypothesis that primary migration is associated with chromatography-like fractionation effects according to polarity. Most importantly, however, the observations from Fig. 1 help to solve the controversy over whether primary migration of oil occurs as a separate phase, or dissolved in water. The major difference in maximum expulsion efficiency between the upper and the lower depletion interval (Fig. 1) supports an oil-phase migration mechanism. Based on the relationships between kerogen-types and hydrocarbon generation^{1,2,8}, it can be deduced that at the time of expulsion the oil saturation of the pore system must have been much higher in the lower source rock interval. More oil was generated there per unit pore volume due to the higher organic carbon content and the superior kerogen quality. In analogy with fluid flow in reservoir sandstones¹³, relative permeability and hence expulsion rates for oil from source rock shales are believed to increase drastically if oil saturation of the pore fluid exceeds ~40% (ref. 14). As this value is clearly in excess of the solubility limit for oil in water (even at the maximum temperature and pressure conditions endured by this sequence) oil must have existed in the pore system as a separate phase in this rich source rock unit. While the expulsion efficiency for the total extract (which is indicative of the total oil) reached a maximum of 80% in the rich, lower source rock interval it reached only a maximum of 30% in the less prolific upper source rock interval. Due to the low concentration of organic carbon and the low hydrocarbon generation potential of the kerogen oil, saturation of the pore system must, even at peak generation stage, have remained very small, resulting in low values of the relative permeability and hence low expulsion rates for the oil.

The increasing expulsion of the saturated hydrocarbons in proximity to the upper and lower shale contact is shown by the

data in Fig. 2. In comparison with the unmodified samples from 1,148.1 and 1,159.5 m, the samples closer to the outer shale contacts exhibit increasingly lower concentrations ($\mu\text{g per g } C_{\text{org}}$) of normal and isoprenoid alkanes. From the upper depletion interval, pristane is expelled to a lesser degree than its *n*-alkane isomer, as revealed by its gradual emergence over the *n*-alkane distribution envelope with increasing depletion. A corresponding effect cannot be observed in the lower depletion interval, because this source rock has, in accordance with its superior kerogen quality (type II), generated a hydrocarbon mixture with a very low relative proportion of pristane.

The compositional fractionation effects associated with expulsion of the saturated hydrocarbons from the upper depletion interval are shown in more detail in Fig. 3. The three samples analysed from the central portion of this source rock interval (1,148.1, 1,152.65 and 1,155.5 m) have the lowest pristane/*n*-heptadecane and pristane/phytane concentration ratios. With an increasing degree of hydrocarbon expulsion into the overlying carrier bed both compound ratios attain higher values, indicating a general depletion trend as shown in Fig. 3. Pristane is expelled less effectively than *n*-heptadecane and phytane, even less so. Furthermore, the ratios for the two thin source rock-type shales (at 1,131.3 and 1,126.7 m) interbedded in the overlying reservoir sandstone unit, and the sample 1,135.3 m near the fracture zone, imply the expected high degrees of hydrocarbon expulsion (Fig. 3). Both parameters have traditionally been used in petroleum geochemistry in a different way: the pristane/*n*-heptadecane ratio to monitor maturity progress¹⁵ and the pristane/phytane ratio to indicate the organic facies¹⁶⁻¹⁸. We have also demonstrated here that regular changes, especially of the pristane/*n*-heptadecane ratio occurring over relatively narrow depth intervals, and independent of changes in kerogen-type, can be used to monitor the degree of hydrocarbon expulsion from the source rocks.

The efficiencies of expulsion of individual *n*-alkanes were quantified for the deeper interval utilizing the concept explained above (Fig. 4). For any given *n*-alkane in the molecular range C_{15} - C_{30} , the expulsion efficiency increases drastically from the slightly depleted samples at 1,160.0 m and 1,160.5 m, to the

moderately depleted one at 1,161.0 m to the highly depleted samples between 1,161.5 and 1,162.6 m close to the lower contact of this shale unit. Among the latter samples the expulsion efficiencies of the *n*-alkanes vary between ~80 and 95%. The higher molecular weight *n*-alkanes have apparently been expelled to a greater extent. However, the trends shown in Fig. 4 are the result of several processes occurring in the sequence: initial hydrocarbon generation–expulsion–replenishment by subsequent hydrocarbon generation. Therefore, these trends could be explained if, after the main phase of expulsion, hydrocarbon generation continued and, due to the high maturity level of this source rock, mainly lower molecular weight alkanes were formed and added to the unexpelled remainder: erroneously low expulsion efficiencies are apparent from Fig. 4 for the lower molecular weight *n*-alkanes. This effect is observed best near the inner edge of the depletion interval (samples at 1,160.0 and 1,160.5 m); due to the more efficient expulsion of the alkanes generated later, the effect is increasingly obliterated towards the outer shale edge.

The values determined in this study for the expulsion efficiencies should not be considered as absolute. The values depend on whether the sample chosen contains the *in situ* generated hydrocarbon concentrations. It is possible that even the samples from the centre of both source rock units have expelled some of their generated oil. Nonetheless, our conclusions remain valid: the values of the expulsion efficiencies are minimum values and the relative differences in the degree of depletion within the shale edges remain the same.

The results of this study have implications for applied petroleum geochemistry. We have shown that, within an individual source rock body, expulsion efficiencies are variable: they change as a function of proximity to the carrier beds and migration avenues and vary with compound-type, molecular size and structure. It may, therefore, be difficult to select approximate mean values for the expulsion efficiencies of entire source rock units so as to calculate the amount of oil which migrated and possibly accumulated in the traps of a given exploration area. Previously reported values for the efficiency of petroleum expulsion from shale source rocks were much lower (10–15%)^{1,2} than the ones documented in this study. However, as previous researchers used a completely different approach (hydrocarbon budget estimates for entire shale and reservoir rock volumes of whole basins), the expulsion efficiency values reported here may not contradict previous experiences. Further work utilizing the approach discussed above should document the natural range of variation of the thicknesses of the depleted edges of average source rock shales at different maturity stages. Another implication of the conclusions reached in this study concerns source rock/crude oil correlation: to establish genetic origins of crude oils more convincingly, an understanding of the nature of hydrocarbon expulsion processes and the associated compositional fractionation effects is required.

We thank the Ruhrkohle AG, Essen, for supplying the sample material, Dr A. S. Mackenzie (BP Research Centre) for a careful review and Dr D. H. Welte (KFA) for support and advice.

Received 19 April; accepted 14 August 1984.

1. Tissot, B. P. & Welte, D. H. *Petroleum Formation and Occurrence* (Springer, Berlin, 1978).
2. Hunt, J. M. *Petroleum Geochemistry and Geology* (Freeman, San Francisco, 1979).
3. Vandenbroucke, M. in *Advances in Organic Geochemistry 1971* (eds Gartner, H. R. & Wehner, H.) (Pergamon, Oxford, 1972).
4. Radke, M., Sittardt, H. G. & Welte, D. H. *Analyt. Chem.* **50**, 663–665 (1978).
5. Radke, M., Willsch, H. & Welte, D. H. *Analyt. Chem.* **52**, 407–411 (1980).
6. Leythaeuser, D. & Schaefer, R. G. in *Advances in Organic Geochemistry 1983* (eds Schenck, P. A., de Leeuw, J. W. & Lijmbach, G. W. M.) (Pergamon, Oxford, in the press).
7. Espitalié, J. et al. *Rev. Inst. Fr. Petrol.* **32**, 23–42 (1977).
8. Tissot, B. P. et al. *Bull. Am. Ass. petrol. Geol.* **58**, 499–506 (1974).
9. Rabinz, A. *Fortschr. Geol. Rheinl. Westf.* **13**, 125–194 (1966).
10. Mackenzie, A. S. et al. *Nature* **301**, 506–509 (1983).
11. Leythaeuser, D. et al. *Bull. Am. Ass. petrol. Geol.* **68**, 196–219 (1984).
12. Altebaumer, A. M., thesis, Univ. Aachen (1983).
13. Craft, B. C. & Hawkins, M. F. *Applied Petroleum Reservoir Engineering* (Prentice-Hall, Englewood Cliffs, 1959).
14. Durand, B. et al. *Proc. 11th World Petroleum Congr.* **1**, 1–13 (1983).
15. Connan, J. & Cassou, A. M. *Geochim. cosmochim. Acta* **44**, 1–23 (1980).
16. Brooks, J. D., Gould, K. & Smith, J. W. *Nature* **222**, 257–259 (1969).
17. Lijmbach, G. W. M. *Proc. 9th World Petroleum Congr.* **2**, 357–369 (1975).
18. Didyk, B. M. et al. *Nature* **272**, 216–222 (1978).

Investigation of claims for interstellar organisms and complex organic molecules

Robert E. Davies*†, Adelaide M. Delluva* & Robert H. Koch†

*Laboratories of Biochemistry, Department of Animal Biology, School of Veterinary Medicine, and † Department of Astronomy and Astrophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

For many years, Hoyle, Wickramasinghe and their associates have examined interstellar (IS) absorption features in the ultraviolet, visible and infrared and 'identified' them with a variety of organic structures or organisms. Among these there have been generalized, pre-biotic molecules¹, polyoxymethylene whiskers², polysaccharides and hydrocarbons^{3,4}, tryptophan (and inferentially proteins)⁵—all claimed to be coatings on IS grains. In other cases, the grains^{1,6} have been described as 10–100% alkanes, alkenes, alkynes or aromatics by mass. In extensions of these claims, the grains are supposed to be microorganisms (such as viruses and bacteria⁷, algae⁸, siliceous cells similar to diatoms⁹, yeasts¹⁰ or other eukaryotic cells¹¹) in whole or in part. Finally, a case has been advanced for possible interstellar and interplanetary insects¹¹. The 'identifications' in these and many other publications call into question the intrinsic origin of Earth life itself^{12,13} and the uniqueness and validity of darwinian evolution¹¹. We now report on ultraviolet spectra of specimens of the types cited by these workers and compare our results with infrared and ultraviolet data published previously. We conclude that the identifications claimed by Hoyle, Wickramasinghe and their colleagues are unwarranted.

The UV results are based on spectrophotometry with the OAO 2 and IUE spacecrafts. Accuracies for these measurements fall in the range ± 5 to $\pm 20\%$ depending on the specific wavelength and star and on the observing circumstances. Speakers at a special meeting of the Royal Astronomical Society¹⁴ noted that IUE spectra may be degraded over extended wavelength intervals (specifically near 280 nm)⁵ and by saturation losses from over-exposure. The visible-band observations concern weak and diffuse absorption bands in the blue through the red and the accuracies of the measurements made on these bands are commonly of the order of $\pm 10\%$. For the IR data beyond 2 μm , accuracy is improving very rapidly and, whether from ground (with its restricted wavelength coverage) or from an airborne observatory, is about $\pm 5\%$. For all these data, spectral resolution ($\Delta\lambda/\lambda$) is of little consequence as the claims of Hoyle, Wickramasinghe and their colleagues all concern relatively broad

Table 1 Comparison of the Karim *et al.* absorption feature with present measurements of tryptophan near 280 nm

Wavelength (nm)	ΔA^*	$A^\dagger$
310	0.00	0.00
300	+0.24	+0.04
290	+0.50	+0.52
280	+0.61	+0.61
270	+0.58	+0.55
260	+0.47	+0.37
250	+0.20	+0.18
240	0.00	+0.12
230	−0.02	+0.91
220	0.00	+3.71
210	+0.01	+2.47
200	+0.02	+2.56
190	+0.02	—
180	+0.02	—

* Karim *et al.*'s excess extinction after subtraction of graphite (0.02- μm radius spheres) extinction.

† Measured extinction due to tryptophan normalized to $A=0.61$ at 280 nm.

Table 2 Observed spectral absorption ratios for specimen materials

Material	λ (nm) [†]	λ' (nm) [‡]	$(A_{\lambda'}/A_{\lambda})$
Tryptophan	280	220	6.1
Bovine serum albumin (BSA)	279	230	4.0
Trypsin (dry)*	279	200	32
RNase (in HCl)*	280	200	63
Aldolase (pH 7.4)*	280	200	50
<i>E. coli</i> (envelope protein in NaHCO ₃)*	275	235	1.4
<i>E. coli</i>	260	220	1.7
<i>E. coli</i> (dry)	260	220	3.1
<i>B. cereus</i> (dry)	260	200	1.4
M13	258	200	27
Nuclear polyhedrosis virus (NPV)	260	200	15
<i>M. luteus</i> DNA	258	200	1.4
Calf thymus DNA	259	200	1.5
<i>Merismopedia</i>	260	200	1.7
<i>Achnanthes brevipes</i>	260	220	2.9
<i>Chlorella</i>	266	200	1.6

* Data taken from ref. 18.

[†] Wavelength in nm of measured peak absorption.[‡] Where $\lambda' > 200$ nm, the sample has an absorbance, > 2.000 , which cannot be recorded accurately at wavelengths ≥ 200 nm.

bands. We have already commented¹⁵ on the treatment of the astronomical data set which Karim *et al.* claimed "confirms" the presence of proteinaceous material in grains⁵. In their Figs 3–5, Karim *et al.* noted the similarity of the absorption spectrum of tryptophan from 310 to 240 nm (including its peak at 280 nm) to a band, nominally freed of the contribution from IS graphite, derived from the ratios of UV spectra of distant, reddened stars to those of nearby comparison stars. Our comments referred to serious mismatches between the paired program and comparison stars and to the reasons why their detailed calculations of the residuum from a particular graphite absorption cannot be defended. However, this property of tryptophan cannot be used alone for identification; this is clear from a perusal of the 118-volume *Sadtler Standard Spectra Collection* which records the UV spectra from 300 to 200 nm of over 34,000 organic compounds. Hundreds of these spectra show peak absorptions with central wavelengths and bandwidths comparable with those of the feature singled out by Karim *et al.*⁵. Furthermore, they represent only a fraction of the several millions of organic compounds now known. Consequently, any particular peak is not specific for any particular molecule, and thus UV spectra alone cannot be used for identification although they may be used to exclude an identification with certainty.

We have, therefore, measured the UV absorbances (which in some cases include the effects of scattering) of some molecules and primitive life forms, including several types which Hoyle and Wickramasinghe have suggested or claimed to exist in IS space.

Absorption spectra were measured accurately from 320 to 235 nm or less in quartz cuvettes (1 cm light path) by means of a Bausch and Lomb Houston Instrument Omniscribe D-5000 strip chart recorder attached to a Varian DMS 90 UV-Visible Spectrophotometer. In all cases except tryptophan, whenever the lowest recorded value was above 200 nm this was because the absorbance had gone off scale (> 2.0) and remained there at all wavelengths down to 200 nm. The region below 240 nm is where the excess absorbance of the Karim *et al.* feature has an absorbance of 0.02 or less. The absorption spectra were obtained for the amino acid tryptophan, the protein bovine serum albumin (BSA), the nucleic acids calf thymus DNA and *Micrococcus luteus* DNA, the bacteriophages M13 and Φ X174, the nuclear polyhedrosis virus (NPV) that attacks the wax moth *Galleria mellonella*, the Gram-positive bacterium *Bacillus cereus*, the Gram-negative bacterium *Escherichia coli* JM 103, the cyanobacterium (blue-green alga) *Merismopedia*, the diatom *Achnanthes brevipes*, and the green alga *Chlorella*. The spectra for tryptophan, BSA, the DNAs, NPV, *E. coli* and *B. cereus*

were also measured on thin films of the dry materials because there would be no free liquid water on interstellar grains.

Table 1 compares our experimental results for tryptophan with the excess extinction from Figs 3 and 5 of Karim *et al.* Disagreement between the two spectra is conspicuous at wavelengths below 240 nm. Our complete results appear in Table 2. For all specimens with an absorption peak in the region 290–250 nm that was different from that at 280 nm, the absorption ratios are even larger when calculated for a standard wavelength of 280 nm.

The results show that, whereas tryptophan and BSA in solution had peaks near 280 nm, every other sample of our materials had a peak near 260 nm that was clearly dominated by the nucleic acids present¹⁶; this does not agree with Karim *et al.*'s expectation⁵ that organisms or their dry remains would have a peak of their UV absorption spectra at or near 280 nm. The effect of drying depended on the size and shape of the particles or crystals formed. For tryptophan, for example, differential scattering can change the position of the observed peak by tens of nanometres and also flatten it conspicuously. In general, absorption peaks are affected by the solvent and its pH—a phenomenon known as 'the universal redshift'¹⁷.

For each one of the wet or dry specimens the absorption band from 210 to 200 nm was higher than the peak between 290 and 250 nm. This finding alone undermines the basis on which Karim *et al.* claimed⁵ to have confirmed the presence of proteinaceous material on IS grains. This claim depended on the almost complete lack of absorption from 240 to 180 nm (see their Fig. 3) by the unknown material they believed responsible for their 280 nm peak. Figure 3 of Karim *et al.*⁵ was normalized at both the 310 nm and 220 nm peaks. This 280 nm peak is the only one within the recorded range of wavelength. It follows that substances or organisms with higher absorbances below 240 nm than at 240 nm must be excluded. This property was found in dry tryptophan and in each of the other materials and organisms tested. For tryptophan in solution, a peak at 220 nm was followed by a shallow trough at 210 nm, then by higher absorbances at and below 200 nm—this particular detail also excludes the identification.

Setlow and Doyle¹⁸ recorded the absorption spectra of dry samples of the protein trypsin and of aldolase and RNase from 310 to 185 nm. All these proteins had absorption peaks at 280 nm but absorbed (and scattered) the incident radiation much more effectively at and below 240 nm. The ratios of the molar absorption coefficients were 32, 50 and 63 for $\epsilon_{200}/\epsilon_{280}$ and 8.6, 12.5 and 13.6 for $\epsilon_{220}/\epsilon_{280}$. These ratios are so large that, if proteins were the source of the absorption, the contribution from graphite would be negligibly small and the curve for HD14250 and its comparison star in Fig. 3 of Karim *et al.* would not have the observed shape.

Although the higher absorption of tryptophan in solution at 230 nm (compared with that at 280 nm) was shown in Fig. 1 of Karim *et al.*⁵, it was neglected in their Fig. 5 which compared "the excess extinction in the star HD14250 relative to graphite" (spheres of 0.02 μ m radius). This comparison was given only from 310 to 245 nm where the curves fit but not for points below 240 nm and especially below 220 nm where the discrepancy becomes increasingly large. A similar objection follows from the papers^{19,20} which Karim *et al.* cite concerning the proteins of tobacco mosaic virus and the envelope proteins of *E. coli*; these papers show a higher absorption for these proteins at or below 240 nm than at 280 nm. This peak is also claimed to confirm the proteinaceous material in grains⁵ from dried bacteria and algae⁸ and viruses¹³.

Karim *et al.* state that "DNA has a strong absorption at 2,600 Å... but the DNA content of microorganisms is so small that prospects of detecting this particular absorption are not encouraging". However, the content of the various ribonucleic acids is very high²¹ and the spectra of the corresponding ribo- and deoxyribonucleotides as well as nucleosides are essentially identical¹⁶. The molar absorption coefficients of the various nucleotides at 260 nm are greater¹⁶ ($\approx 9,000$ to $\approx 15,000$) than

that of tryptophan ($\approx 5,000$) at 280 nm. Furthermore, tryptophan comprises only 3 of the 336 amino acids in each *E. coli* envelope protein, but 25 of the amino acids are tyrosine and this moves the peak absorption to 275 nm (ref. 20). This accounts for *E. coli* and the other organisms enumerated above having peaks near 260 nm rather than 280 nm.

It is well known that the sterilization action spectrum of UV radiation from 300 to 220 nm parallels the absorption spectrum of bacteria^{22,23} and that enzymes are inactivated by UV-induced damage to phenylalanine, tyrosine, tryptophan and cystine²⁴. If tryptophan and these other amino acids were exposed to the interstellar UV, they would soon be destroyed. For example, the quantum yield for the production of ammonia, by 253.7-nm radiation, from tryptophan is 0.002 (ref. 24). If the amino acids were protected deep inside a grain covered by a polymeric coat, they could not be observed. However, a literature search failed to uncover any finding of tryptophan or any other indole derivative in either meteorites or IS clouds. One paper¹⁰ concludes that the IR spectrum of the galactic centre source IRS7 contains strong evidence for bacterial grains on the basis of the IR spectrum of *E. coli*. However, from these results we have calculated that the same bacterial mass would cause an absorbance ≈ 100 times larger in the UV than in the IR and this has not been observed in the direction to any star. The same comparison excludes polysaccharides^{25,26} associated with diatoms⁹ and algae⁸. Finally, on the basis of experiments of Rosenbusch²⁰ and Setlow and Doyle¹⁸, pure proteins in general^{5,10} (including the major envelope protein of *E. coli*²⁰) have a maximum UV absorbance at least 100 times larger than the highest IR absorbance. Because neither the required shape nor magnitude of the UV absorbances are observed, proteins are also excluded as explanation for these absorbances.

After this work was completed, Williams²⁷ and Hoyle and Wickramasinghe²⁸ published letters also describing the recent adverse comment¹⁴ on the conclusions of Hoyle, Wickramasinghe and colleagues. Turner²⁹ expressed the need for much more stringent requirements than have recently been applied for molecular identification in the microwave region; although he is concerned only with highly resolved spectra, the same constraints apply even more forcibly to presently resolvable IR and UV molecular bands.

The M13 and *E. coli* JM 103 were gifts from Dr Roselyn Eisenberg; the DNA samples from Dr Phoebe S. Leboy; the other viruses and living organisms came from the Carolina Biological Supply Co., North Carolina.

Note added in proof: Greenberg's claim (see ref. 14) that the 280 nm feature⁵ is spurious has now been published³⁰ together with summaries of papers given at the Royal Astronomical Society's Specialist Discussion, 'Are Interstellar Grains Bacteria?'³¹. Other adverse comments have appeared³²⁻³⁵. The response to these by Karim, Hoyle and Wickramasinghe^{36,37} has in turn been answered by us³⁵.

Received 3 January; accepted 15 August 1984.

1. Hoyle, F. & Wickramasinghe, N. C. *Nature* **266**, 241-243 (1977).
2. Wickramasinghe, N. C. *Mon. Not. R. astr. Soc.* **170**, 11P-16P (1975).
3. Hoyle, F. & Wickramasinghe, N. C. *Nature* **270**, 323-324 (1977).
4. Hoyle, F. & Wickramasinghe, N. C. *Mon. Not. R. astr. Soc.* **181**, 51P-55P (1977).
5. Karim, L. M., Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **94**, 223-229 (1983).
6. Wickramasinghe, D. T. & Allen, D. A. *Nature* **287**, 518-519 (1980).
7. Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **65**, 241-244 (1979).
8. Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **66**, 77-90 (1979).
9. Hoyle, F., Wickramasinghe, N. C. & Al-Mufti, S. *Astrophys. Space Sci.* **86**, 63-69 (1982).
10. Hoyle, F., Wickramasinghe, N. C., Al-Mufti, S., Olavesen, A. H. & Wickramasinghe, D. T. *Astrophys. Space Sci.* **83**, 405-409 (1982).
11. Hoyle, F. & Wickramasinghe, N. C. *Evolution from Space*, 151-159 (Simon & Schuster, New York, 1981).
12. Hoyle, F. & Wickramasinghe, N. C. *Lifecloud The Origin of Life in the Universe*, 157-165 (Harper & Row, New York, 1978).
13. Hoyle, F. & Wickramasinghe, N. C. *Diseases from Space*, 1-11 (Harper & Row, New York, 1979).
14. Campbell, P. *Nature* **306**, 218-219 (1983).
15. Koch, R. H. & Davies, R. E. *Astrophys. Space Sci.* **100**, 425-426 (1984).
16. Lehninger, A. L. *Biochemistry* 2nd edn, 314 (Worth, New York, 1975).
17. Weber, G. & Teale, F. W. J. in *The Proteins* Vol. 3, 2nd edn (ed. Neurath, H.) 445-521 (Academic, New York, 1965).
18. Setlow, R. & Doyle, B. *Biochim. biophys. Acta* **24**, 27-41 (1957).
19. Taniguchi, M., Yamaguchi, A. & Taniguchi, T. *Biochim. biophys. Acta* **251**, 164-171 (1971).
20. Rosenbusch, J. P. *J. biol. Chem.* **249**, 8019-8029 (1974).

21. Feinberg, G. & Shapiro, R. *Life Beyond Earth*, 55 (Morrow, New York, 1980).
22. Gates, F. L. *J. gen. Physiol.* **14**, 31-42 (1930).
23. Davis, B. D., Dulbecco, R., Eisen, H. N. & Ginsberg, H. S. *Microbiology* 3rd edn, 1267 (Harper & Row, New York, 1980).
24. Setlow, J. K. *Compreh. Biochem.* **27**, 157-209 (1967).
25. Hoyle, F. & Wickramasinghe, N. C. *Nature* **268**, 610-612 (1977).
26. Hoyle, F., Olavesen, A. H. & Wickramasinghe, N. C. *Nature* **271**, 229-231 (1978).
27. Williams, D. A. *Nature* **306**, 420 (1983).
28. Hoyle, F. & Wickramasinghe, N. C. *Nature* **306**, 420 (1983).
29. Turner, B. E. *Astrophys. Lett.* **23**, 217-224 (1983).
30. Greenberg, J. M. *Observatory* **104**, 134-135 (1984).
31. *Observatory* **104**, 129-139 (1984).
32. McLachlan, A. & Nandy, K. *Observatory* **104**, 29-31 (1984).
33. Whittet, D. C. B. *Observatory* **104**, 159-160 (1984).
34. Savage, B. D. & Sitko, M. L. *Astrophys. Space Sci.* **100**, 427-429 (1984).
35. Duley, W. W. Q. *J. R. astr. Soc.* **2**, 109-113 (1984).
36. Karim, L. M., Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **100**, 431-435 (1984).
37. Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **103**, 189-193 (1984).
38. Davies, R. E., Delluva, A. M. & Koch, R. H. in *The Search for Extraterrestrial Life—Recent Developments* (ed. Papagiannis, M. D.) Int. ast. Un. Symposium 112 (Reidel, Dordrecht, in the press).

Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation

Kam Hui, Frank Grosveld* & Hilliard Festenstein

Department of Immunology, The London Hospital Medical College, Turner Street, London E1 2AD, UK

* Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Major histocompatibility complex (MHC) class I molecules can function as specific target antigens in T-cell-mediated cytotoxicity^{1,2}. In addition, T cells can kill target cells through non-MHC antigens, for example, virally infected cells, if the target and effector cells express the same MHC class I antigens². Consequently, quantitative and/or qualitative variations in the expression of the H-2/HLA antigens on the target cells could interfere with MHC-restricted immune reactions. We have reported that the AKR leukaemia cell line K36.16, a subline of K36 (ref. 3), on which the H-2K^k antigen cannot be detected, is resistant to T-cell lysis and grows very easily in AKR mice⁴. Other AKR tumour cell lines, like 369, which have a relatively large amount of H-2K^k on their surface, are easily killed by T cells *in vitro* and require a much larger inoculum to grow *in vivo*⁴. Monoclonal antibodies against H-2K^k, but not against H-2D^k, prevented the killing by T cells^{4,5}. This suggests that some tumour cells grow *in vivo* because tumour-associated antigen(s) cannot be recognized efficiently by the host's immune system, due to the absence of MHC molecules which would function as restriction elements for T-cell cytotoxicity. We have tested this hypothesis by introducing the H-2K^k gene into the H-2K^k-deficient AKR tumour cell line K36.16 and have now demonstrated directly the biological relevance of H-2K^k antigen expression in the regulation of the *in vivo* growth of this tumour cell line.

Transformations were done by calcium phosphate co-precipitation²⁰ with a cosmid containing the H-2K^k gene (c27.2) and pTCF⁶, the vector for the aminoglycosyl 3'-phosphotransferase gene (*agpt*), or pTCF alone. Between 8 and 20 transformants per 10⁶ cells were selected in medium containing 800 $\mu\text{g ml}^{-1}$ of the antibiotic G418. From over 300 transformants, we selected a series of clones that expressed different amounts of H-2K^k on the cell surface (see below). DNA and RNA were isolated from each of the selected transformants and analysed by Southern blot or S₁ nuclease protection analysis. Hybridization with a plasmid (vector) or an H-2K^k probe showed that each of the transformants contained one to three copies of the exogenous H-2K^k gene (data not shown). Figure 1 shows the level of H-2K^k mRNA determined by S₁ nuclease protection assay in the K36.16 tumour cells before and after transformation with H-2K^k. The negative control H-2K^d P₃-X63-Ag8 (Ag8) cells⁷ (Fig. 1b) and the K36.16 cells show a low mRNA signal, probably due to incomplete S₁ digestion of RNA-DNA hybrids

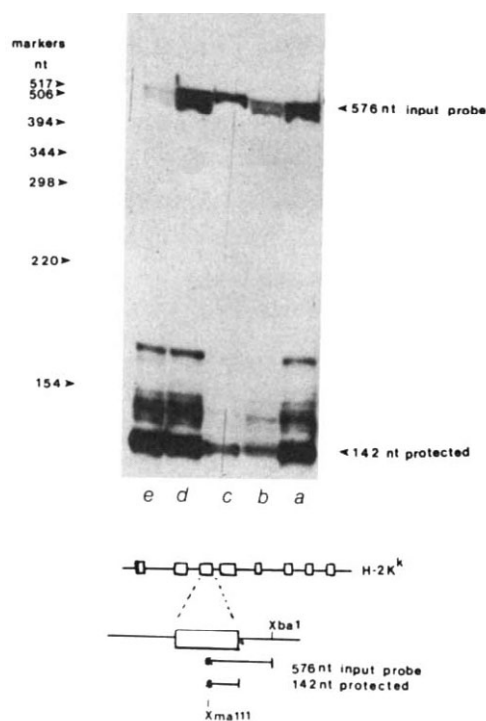


Fig. 1 S_1 nuclease protection analyses of mRNA isolated from the c27.2 transformants and control cell lines: *a*, 369 ($H-2K^k$, positive control); *b*, Ag8 ($H-2K^d$, and $H-2K^k$ -negative control); *c*, K36.16; *d*, C27.2.2; and *e*, C27.2.3.

Methods: S_1 nuclease protection analysis was done using procedures described elsewhere^{18,19}. The input probe is a 576-nucleotides (nt) *XmaIII-XbaI* DNA fragment isolated from the third exon-intron boundary; 142 nt of this fragment are protected from S_1 nuclease digestion (S. Kvist, personal communication). 25 μ g of total cytoplasmic RNA isolated from the different cell lines were hybridized overnight at 57 °C with the labelled DNA input probe. S_1 nuclease cleavage was at 37 °C for 2 h and electrophoretic separation was on 8 M urea-7% polyacrylamide gels, resulting in a 142-nucleotide protected fragment. Multiple protected bands shorter than 142 nt are due to overdigestion by S_1 nuclease.

formed between the DNA probe and mRNA molecules other than $H-2K^k$. The $H-2K^k$ -transformed cells, however, show an increase of at least 20-fold of $H-2K^k$ mRNA (Fig. 1*d, e*) to a level similar to that of the positive control, 369 ($H-2K^k$ -positive) (Fig. 1*a*). This suggests that the absence of the $H-2K^k$ antigen on the K36.16 cells is due to a failure to transcribe the $H-2K^k$ gene efficiently, rather than any post-transcriptional event.

To demonstrate the presence of the $H-2K^k$ antigen on the cell surface, we carried out radiobinding analysis with anti- $H-2K^k$ monoclonal antibodies. Table 1 gives the results from only five $H-2K^k$ DNA transformants and the control cell lines, because they cover a wide range of $H-2K^k$ antigen expression on the cell surface and were subsequently used in experiments designed to evaluate the role of $H-2K$ molecules in oncogenesis and host resistance to AKR leukaemias. All these cell lines express the same amount of $H-2D^k$, as expected (data not shown). In addition, a 45,000-molecular weight molecule was precipitated by anti- $H-2K^k$ monoclonal antibody from 35 S-methionine-labelled lysates of one of the transformed clones. Competition by added unlabelled AKR ($H-2K^k$ -positive) lysates specifically prevented the immunoprecipitation of this molecule (data not shown).

Because the $H-2K^k$ antigen is important as a restriction element in T-cell-mediated killing of Gross virus tumours⁴, we examined the ability of these transformed clones to induce tumours in syngeneic AKR and semi-syngeneic (AKR $\times$ BALB/c)F1 mice. Table 1 shows that inocula of 10^5 viable cells

Table 1 Radioimmunoassay and tumour inducibility of $H-2K^k$ -transfected K36.16 cells

Transformant	Radiobinding (net c.p.m.)	No. of mice with tumours*
K36.16	0 (0)	10/10 (10/10)
pTCF-K36.16	92 (21)	10/10 (10/10)
C27.2.1	364 (424)	0/10 (0/10)
C27.2.2	376 (529)	1/5
C27.2.3	1,334 (1,073)	0/5 (0/5)
C27.2.4	257 (209)	4/5
C27.2.5	230 (497)	0/5
369	19,416 (6,350)	

For radiobinding, 5×10^5 cells were reacted with monoclonal antibodies as indicated. The anti- $H-2K^k$ (refs. 15, 16) and anti- $H-2D^k$ (ref. 15)-producing hybridoma cell lines were obtained from the American Type Culture Collection. The results are an average of two independent assays with anti- $H-2K^k$ antibody 11.4.1 (ref. 15) (or 16-3-22S¹⁶) and are expressed as c.p.m. after subtraction of the background (200 c.p.m.) pTCF-K36.16 is a clone obtained by transformation of K36.16 with the vector pTCF alone. The positive control, 369, is an AKR leukaemia that expresses $H-2K^k$ antigen⁴.

* 100,000 K36.16 tumour cells (in 0.1 ml phosphate-buffered saline, PBS) were injected subcutaneously (s.c.) into one thigh and the same number of c27.2 transfected clones into the other thigh of the recipient mouse. The diameters of the tumour masses were measured after 3 weeks. Mice were scored as positive for tumours when the diameters measured were 1.5–2.0 cm, whereas mice were scored as negative for tumours when the diameters observed were <0.6 cm. 3,000 K36.16 tumour cells were able to induce 100% tumour incidence in both AKR and (AKR $\times$ BALB/c)F1 mice in 14–20 days. The values for F₁ mice are given in parentheses.

Table 2 Induction of tumour by treatment of the transfected clones with monoclonal anti- $H-2K^k$ antibody

Clone	Treatment of AKR recipients	No. of mice with tumours
C27.2.1	Anti- $H-2K^k$ MoAb	3/5
C27.2.1	Anti- $H-2K^b$ MoAb	0/5
C27.2.1	Culture medium	0/5
C27.2.3	Anti- $H-2K^k$ MoAb	6/7
C27.2.3	Anti- $H-2K^b$ MoAb	0/7

100,000 transfected clones treated with monoclonal antibody (MoAb) (anti- $H-2K^k$ MoAb 16-3-22S¹⁶ or anti- $H-2K^b$ MoAb B8-24-3 (ref. 17) as indicated were injected s.c. into the thigh of one of the hind legs of the recipient, and K36.16 cells treated with the same antibody were injected into the other hind leg of the same mouse. This was followed by subsequent injections of the corresponding monoclonal antibody *in vivo* once every other day. The number of tumour incidences was recorded after 20 days. The K36.16 tumour cells were able to induce tumours in all cases.

Table 3 Mice immunized with the transfected clones reject a second challenge of the original K36.16 tumour cells

Mouse strain tested	Mice primed with clone	No. of mice with tumours
AKR	C27.2.3	0/5
AKR	C27.2.3 (mitomycin C)	4/5
(AKR $\times$ BALB/c)F1	C27.2.3	0/5
AKR	C27.2.2	0/5
AKR	C27.2.4	0/2
AKR	K36.16 (mitomycin C)	5/5

Mice were injected s.c. with 100,000 c27.2 transfected cells as indicated in Table 1. After 3–4 weeks, mice that did not show signs of tumours were given an additional dose (s.c.) of 100,000 K36.16 tumour cells. The number of mice with tumours was observed after 30 days. The K36.16 (mitomycin C) cells were treated with mitomycin C at $50 \mu\text{g ml}^{-1}$ 10^6 cells for 30 min at 37 °C, washed four times with PBS and subsequently injected s.c.

of the original K36.16 tumour cells and a clone of K36.16 tumour cells transformed with the vector pTCF alone, induced necrotic tumours within 20 days. However, inocula of 10^5 viable cells each of clones C27.2.1, C27.2.3 and C27.2.5 all failed to induce tumours in AKR and (AKR $\times$ BALB/c)F1 mice; these clones were able to induce tumours in all cases when the recipient AKR mice were irradiated (700 rad) before injection. Clones C27.2.2 and C27.2.4, which express relatively lower amounts of H-2K^k antigens (Table 1), were both able to induce tumours in some of the AKR mice. Interestingly, the diameters of the tumour masses which developed after inoculation of the latter clones were smaller (0.9–1.2 cm) and necrosis was absent even after 30 days. The oncogenicity of these transformed clones may thus be determined by: (1) the level of expression of the H-2K^k gene product in a particular transformant; (2) the ability of individual recipient mice to mount an efficient immune response against these inocula; and (3) the size of the inoculum needed to induce a tumour. When a higher dose of 5×10^6 viable cells was given, C27.1.1 and C27.2.3 were able to develop tumours in AKR mice (C27.2.5 was not tested). Conversely, clones C27.2.2 and C27.2.4 failed to induce tumours in AKR mice if a lower dose of 5×10^3 cells, instead of 10^5 cells, was given.

To prove that H-2K^k is the restriction element needed for the *in vivo* rejection of the transformed clones, C27.2.1 and C27.2.3 were first allowed to react separately with several different monoclonal antibodies (as shown by Table 2) before injection into the mice. The corresponding monoclonal antibodies were subsequently injected once every other day for 5 days. This procedure enabled the transformed clones to induce tumours in most AKR recipients (three out of five for C27.2.1, and six out of seven for C27.2.3; Table 2). Injection of the irrelevant anti-H-2K^b monoclonal antibody and culture medium were both ineffective.

To test whether the H-2K^k DNA-transformed clones can confer immunity, mice were injected with either the transformed clones, or mitomycin C-treated K36.16 tumour cells or C27.2.3 cells as indicated in Table 3. After 3 weeks, mice that did not show signs of tumours were again challenged with an additional 10^5 live K36.16 tumour cells. While only 3×10^3 K36.16 tumour cells always produced a tumour, the mice which had rejected the H-2K^k-transformed K36.16 clones were protected against a subsequent challenge of 10^5 live K36.16 tumour cells—this even included mice which had rejected the relatively low H-2K^k-expressing clone C27.2.4. Thus, once an efficient immune response was obtained, this protective immunity was as efficient as that elicited by the more immunogenic clones (for example, C27.2.3) against a subsequent challenge of the original K36.16 tumour cells. When the H-2K^k-positive C27.2.3 cells were prevented from dividing by treatment with mitomycin C before injection, their ability to induce protection against a second challenge of live K36.16 tumour cells was abrogated (Table 3).

These experiments raise several interesting questions. First, the importance of the expression of the H-2K^k product seems to be central to the host response to tumours. The examination of many AKR tumours showed that several of them had only a small amount of H-2K^k antigen on the surface⁴. These findings and those of others^{8–11} suggest that the relative absence of the H-2K specificity provides such tumours with a selection advantage *in vivo*^{12,13}. One might expect, as indeed is found, that the cell-mediated cytotoxicity of tumours which are H-2D-restricted would exhibit a relative absence of the H-2D-encoded products¹⁴.

The results presented here strongly suggest that the selective loss of the H-2K^k restriction elements in K36.16 is caused by a 'switch-off' at the transcriptional level, rather than any changes in the amino acid sequences or any fault in the mechanism necessary for the insertion of the antigens in the cell membrane. Experiments to study the molecular basis of this suppression are in progress.

We thank M. Steinmetz for the cosmid c27.2; S. Kvist for providing unpublished information in preparing the H-2K^k DNA probe for the S₁ nuclease protection analysis; and C.

O'Carroll for typing the manuscript. The '369' tumour line was provided by Peter Krammer. These studies were supported by the CRC and the MRC (UK).

Received 26 July; accepted 3 September 1984.

- Schreffler, D. C. & David, C. S. *Adv. Immun.* **20**, 125–195 (1975).
- Zinkernagel, R. M. & Doherty, P. D. *Adv. Immun.* **27**, 52–177 (1979).
- Old, E. J., Boyse, E. A. & Stockert, E. *Cancer Res.* **25**, 813–819 (1965).
- Festenstein, H. & Schmidt, W. *Immun. Rev.* **60**, 85–127 (1981).
- Schmidt, W. & Festenstein, H. *Immunogenetics* **16**, 257–264 (1982).
- Grosfeld, F. G. *et al. Nucleic Acids Res.* **10**, 6715–6732 (1982).
- Horbita, K. & Harris, A. W. *Exp. Cell Res.* **60**, 61–70 (1970).
- Baldani, P., Pogo, F., Gisselbrecht, S. & Komilsky, P. *J. exp. Med.* **158**, 1294–1306 (1983).
- Eisenbach, L., Segal, S. & Feldman, M. *Int. J. Cancer* **32**, 113–120 (1983).
- Gooding, L. R. *J. Immun.* **129**, 1306–1312 (1982).
- Rogers, M. J., Gooding, L. R., Margulies, D. H. & Evans, G. A. *J. Immun.* **130**, 2418–2422 (1983).
- Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. *Nature* **305**, 771–775 (1983).
- Bernards, R. *et al. Nature* **305**, 776–779 (1983).
- Pierotti, M. A., Ballina, D., Colombo, M. P., Gragiol, L. & Parmiani, G. *Transplant. Proc.* **15**, 2068–2073 (1983).
- Oi, V., Jones, P. P., Goding, J. W. & Herzenberg, L. A. *Curr. Topics Microbiol. Immun.* **81**, 115–129 (1978).
- Ozata, K., Mayer, N. & Sachs, D. H. *J. Immun.* **124**, 533–540 (1980).
- Köhler, G., Fischer Lindahl, K. & Hensser, C. *Immune System* **2**, 202–208 (1981).
- Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
- Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
- Wigler, M. *et al. Cell* **16**, 777–785 (1979).

Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells

Stephen V. Desiderio*, George D. Yancopoulos†, Michael Paskind*, Elise Thomas*‡, Michael A. Boss*‡, Nathaniel Landau*, Frederick W. Alt† & David Baltimore*

* Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

† Columbia University, College of Physicians and Surgeons, Department of Biochemistry, New York, New York 10027, USA

The variable regions of immunoglobulin heavy chains are encoded in the germ line by three discrete DNA segments: V_H (variable) elements, D (diversity) elements and J_H (joining) elements. During the differentiation of B lymphocytes, individual segments from each group are brought together by recombination to form the complete V_HDJ_H variable region^{1–8}. To understand these processes better, we have now isolated and sequenced molecular clones representing intermediates (DJ_H fusions) and final products (V_H-to-DJ_H joins) of heavy-chain gene rearrangement in two cell lines^{9,10} that represent analogues of cells at early stages of B-lymphocyte differentiation^{11–13}. Heavy-chain gene assembly in one cell line but not in the other is accompanied by the appearance of short nucleotide insertions at the recombinational junctions. The generation of such insertions is positively correlated with the expression of terminal deoxynucleotidyl transferase in these lines.

The two cell lines used in our study, 40E4 and 22D6, were produced by Abelson murine leukaemia virus (A-MuLV) transfection of BALB/c fetal liver at 13–14 and 17–19 days of gestation, respectively. These cell lines produce no detectable immunoglobulin¹²; they possess DJ_H rearrangements on both chromosomes and undergo further rearrangement during propagation^{12–14}. Thus the 40E4 and 22D6 cell lines seem to represent the stage of differentiation during which lymphoid progenitor cells give rise to μ -positive pre-B cells.

Two clonal cell lines, 40E4-2 and 22D6-G, were isolated, carried in culture for about 25 generations and subcloned to generate sets of secondary clonal isolates. The clonal origin of these isolates was verified by analysis of the pattern of A-MuLV

‡ Present addresses: Tufts University, School of Veterinary Medicine, Boston, Massachusetts 02111, USA (E.T.); Celltech Ltd, Slough SL1 4DY, UK (M.A.B.).

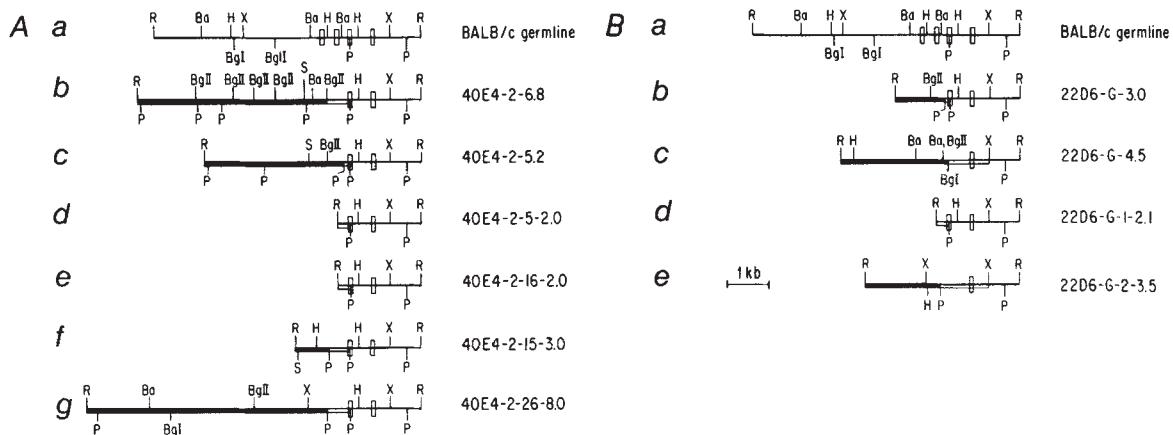


Fig. 1 Restriction maps of molecular clones representing J_H -associated rearrangements in derivatives of 40E4-2 and 22D6-G. **A**, Partial restriction maps of recombinants from 40E4-2 and its derivatives. **a**, Map of the 6.2-kb J_H -containing *EcoRI* fragment from the BALB/c germ line³, shown for comparison. **b–f**, Restriction maps of the following J_H -associated rearrangements: **b**, 40E4-2-6.8; **c**, 40E4-2-5.2; **d**, 40E4-2-5-2.0; **e**, 40E4-2-16-2.0; **f**, 40E4-2-15-3.0; **g**, 40E4-2-26-8.0. Narrow lines represent regions of identity with the BALB/c germ-line map. Closed bars represent the minimal extent of novel DNA sequence; open bars designate the localization of the recombinant junctions. Restriction enzymes are abbreviated as follows: R, *EcoRI*; Ba, *BamHI*; H, *HindIII*; X, *XbaI*; P, *PstI*; Bgl, *BglI*; BglI, *BglII*; S, *SacI*. **B**, Partial restriction maps of recombinants from 22D6-G and its derivatives: **a**, the BALB/c 6.2-kb germ-line *EcoRI* fragment; **b**, 22D6-G-3.0; **c**, 22D6-G-4.5; **d**, 22D6-G-1-2.1; **e**, 22D6-G-2-3.5. Regions of novel DNA sequence and locations of recombinant junctions are identified as in **A**.

Methods: Genomic DNA libraries for each of the A-MuLV-transformed parental cell lines and their derivatives were constructed in Charon 16A and screened for hybridization to a J_H probe¹⁴. J_H -containing bacteriophage insert fragments were subcloned into the *EcoRI* site of pBR322 and partial restriction maps were determined. The A-MuLV-transformed cell lines were produced as described by Rosenberg and Baltimore⁹ and Waneck and Rosenberg¹⁰. Cell lines were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf-serum and 50 μ M 2-mercaptoethanol. Primary subclones 40E4-2 and 22D6-G were derived from the 40E4 and 22D6 cell lines by limiting dilution to a concentration of 0.2 cells per microtitre well. These primary subclones were carried for 2 weeks in culture and secondary subclones were obtained by limiting dilution as described above. Preparation of genomic DNA, restriction enzyme digestion, agarose gel electrophoresis, radiolabelling of probes by nick-translation and DNA hybridization were performed as previously described²⁹. Genomic DNA libraries were constructed in the bacteriophage vector Charon 16A³⁰. Genomic DNA from 40E4-2, 40E4-2-26, 22D6-G or 22D6-G-2 was digested with *EcoRI* and ligated to Charon 16A DNA, which had previously been digested with *EcoRI* and dephosphorylated with calf intestinal phosphatase. Genomic DNA from 40E4-2-5, 40E4-2-16 or 22D6-G-1 was cut with *EcoRI* and fractionated on gradients of 10–40% sucrose containing 1 M NaCl, 20 mM Tris-Cl pH 8.0 and 10 mM EDTA. Centrifugation was performed at 24,000 r.p.m. and 20 °C for 24 h in the SW27 rotor. Fragments <2.5 kb long were selected from digests of 40E4-2-5, 40E4-2-16 and 22D6-G-1 genomic DNA; fragments <3.5 kb long were selected from the digest of 40E4-2-15 genomic DNA. These size-selected pools were ligated to Charon 16A DNA, prepared as described above. The products of ligation were encapsidated *in vitro* by the method of Sternberg *et al.*³¹. The resultant bacteriophage were propagated in *Escherichia coli* strain LE392. Plaques were screened for hybridization to the ³²P-labelled J_H probe by the method of Benton and Davis³². Bacteriophage positive for hybridization were isolated by three additional rounds of plaque purification. After initial propagation in Charon 16A, the J_H -containing *EcoRI* fragments were subcloned into the *EcoRI* site of pBR322.

proviral integration¹³, which was identical among all members of each set of subclones (data not shown). Hybridization of J_H and *D* probes to DNA from both series of clonal cell lines showed that each parental line had two DJ_H rearrangements and that many subclones had lost one or both DJ_H -containing fragments but had acquired new fragments¹⁴. The new fragments generally hybridized well to the J_H probe but not to the *D* probe, suggesting that they might have arisen by appendage of V_H segments onto pre-existing DJ_H units.

To determine the structures of the J_H -associated rearrangements present in the 40E4-2 and 22D6-G cell lines and their derivatives, we carried out molecular cloning of each putative V_H -to- DJ_H rearrangement. The restriction maps of the fragments and their relationship to the J_H region of BALB/c germ-line DNA are shown in Fig. 1. In the 40E4-2 series, the original DJ_H rearrangements were near J_H3 and further rearrangements also occurred near J_H3 . In 22D6-G, one rearrangement was near J_H4 and the other near J_H3 ; the subclones had secondary rearrangements near the same regions. The areas around these points of rearrangement were therefore chosen for DNA sequence analysis.

From its sequence, it is evident that the 5.2-kilobase (kb) J_H -containing *EcoRI* fragment from the 40E4-2 line had arisen by recombination between an SP2-type *D* segment⁸ and J_H3 (Fig. 2A, *a–c*). The 5' flank of the *D* region and part of its coding sequence are totally nonhomologous to $D_{SP2.7}$ but, as is often found at DJ_H joints^{2,5–8}, some nucleotides were apparently lost from both the *D* and J_H potential coding sequences. Also, 7 nucleotides not represented in the $D_{SP2.7}$ or J_H3 germ-line sequences were found between *D* and J_H ; they constitute an *N* region¹⁶.

A similar analysis of the sequence in the 6.8-kb allele showed that it, too, consisted of part of *D*, part of J_H3 and an *N* region of 4 nucleotides (Fig. 2B). The exact germ-line sequence for this allele is not known, although it is most similar to $D_{SP2.3}$ (see Fig. 2B).

The alleles cloned from 22D6-G had also lost some coding sequence from J_H or *D* (Fig. 3A, *a–c*; B, *a–c*). Both *D* regions were identical to known germ-line *D* segments. The rearrangements differed from rearrangements in 40E4-2 in that all of the nucleotides near the joints could be accounted for by *D* and J_H germ-line sequences; no insertion of *N* regions seemed to have occurred.

The presence of the highly conserved¹⁷ 3' end of the V_H segment in all four secondary rearrangements of the 5.2-kb DJ_H -containing *EcoRI* fragment in 40E4-2 and both rearrangements in 22D6-G shows that all of these rearrangements involved recombination of V_H segments with pre-existing DJ_H segments (Figs 2A, *e–h*; 3Ae, Bd). All six of these V_H -to- DJ_H rearrangements have used closely related members of the V_H7183 gene family, of which the MOPC21 V_H gene is representative¹⁸. In all but one case, whenever a new band appeared in a subclone, the new fragment contained the DNJ_H structure characteristic of the fragment that had been lost (see Figs 2, 3), proving that a pre-existing DJ_H gives rise to a V_HDJ_H unit.

The junctions formed during V_H -to- DJ_H joining were similar in several respects to the junctions formed during *D*-to- J_H joining. First, there was loss of a variable number of nucleotides from the 5' end of the DJ_H coding sequence. Second, variable numbers of nucleotides were lost from the 3' ends of the V_H coding sequences. This was definitively observed in the

Fig. 2 V_H - DJ_H assembly in the 40E4-2 A-MuLV transformant. **A**, Primary and secondary rearrangement of the 5.2-kb J_H -containing *EcoRI* fragment from 40E4-2. Recombinant junctions in molecular clones of the primary (5.2 kb) and secondary rearrangements were localized by restriction mapping, and nucleotide sequences across the joints were determined by the method of Maxam and Gilbert²³.

a, b, Partial sequences of the germ-line J_H3 segment^{3,7} and $D_{SP2.7}$ segments⁸. **c**, Sequence of the $D_{SP2.7}$ - J_H3 fusion in clone 40E4-2-5.2. **d**, 3'-Terminal portion of the germ-line sequence of the V_H81X gene segment¹⁸. **e, f**, Sequences at the recombinant junctions of clones 40E4-2-5-2.0 and 40E4-2-16-2.0; these recombinants resulted from joining of V_H81X to the D - J_H3 fusion of 40E4-2-5.2. **g, h**, Sequences at the recombinant junctions of clones 40E4-2-15-3.0 and 40E4-2-26-8.0; these rearrangements are also the result of V_H -to- DJ_H joining. Sequences derived from J_H coding regions are indicated by a dashed underline; sequences derived from D regions are given in boldface type. Conserved heptamer and nonamer recognition sequences are indicated. Nucleotide regions not accounted for by germ-line V_H , D or J_H sequences (N regions) are enclosed in boxes and labelled N .

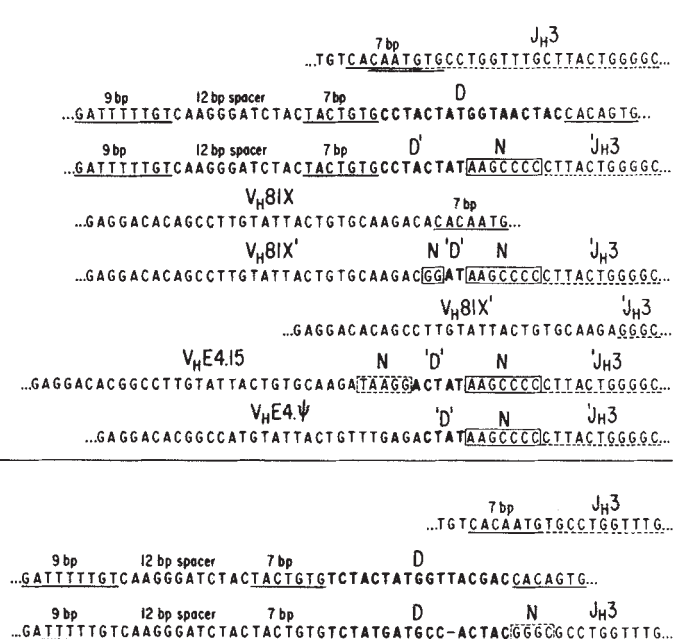
Dotted boxes designate probable N regions in instances where the precise germ-line sequence of one recombination partner has not been determined (see text). Loss of nucleotides from the 5' or 3' side of a coding segment is designated by 'X' or 'X'', respectively. **B**, Primary rearrangement of the 6.8-kb J_H -containing *EcoRI* fragment from 40E4-2. The recombinant junction in the 6.8-kb fragment was localized by restriction mapping and the nucleotide sequence across the joint was determined as above. **a, b**, Partial sequences of the germ-line J_H3 and $D_{SP2.3}$ segments⁸. **c**, Sequence of the D - J_H3 fusion in clone 40E4-2-P-6.8. The D segment used is not identical to any known D segment, although it is similar to $D_{SP2.3}$. The hyphen indicates a gap introduced in the D coding region to maximize homology to $D_{SP2.3}$. Symbols used are defined in **A**.

recombinants 40E4-2-5-2.0 and 40E4-2-16-2.0 both of which used the V_H81X segment¹⁸ and lost one and two nucleotides, respectively, from its 3' end (Fig. 2Ad-f). Comparison with related V_H sequences¹⁷ suggests that the V_H segment used in 40E4-2-15-3.0 has probably lost no nucleotides whereas the V_H segment used in 40E4-2-26-8.0 has lost at most one (Fig. 2Ag, h).

A third characteristic of V_H -to- DJ_H joining was that nucleotides not present in the germ-line sequences appeared at the V_H - DJ_H junction in at least one and probably two of the four secondary recombinants derived from 40E4-2. In 40E4-2-5-2.0, two dG residues appeared at the boundary between V_H81X and D , neither of which was present in the germ-line V_H81X segment or the parental DJ_H segment. Using related V_H segments¹⁷ for comparison, it seems likely that five nucleotides (TAAGG) had been introduced at the V_H - DJ_H junction in 40E4-2-15-3.0 which were not encoded by either V_H or the parental DJ_H segment.

As in the 40E4-2 derivatives, the V_H - DJ_H junctions in the 22D6-G subclones exhibited variable loss of nucleotides from the V_H and DJ_H coding segments. In neither recombinant, however, did we find nucleotides at the V_H - DJ_H junction that could not be accounted for by the DJ_H or V_H sequences.

Comparison of the V_HDJ_H segments found in derivatives of 40E4-2 and 22D6-G revealed one obvious difference: recombinants from the 40E4-2 line commonly contained extra nucleotides not encoded by V_H , D or J_H segments whereas recombinants from the 22D6-G line did not. These N regions, which have also been found in DJ_H and V_HDJ_H fusions from terminally differentiated cell lines^{5,6,8,16}, vary widely in length and sequence and it therefore seems unlikely that each is encoded in the germ line. It has been suggested that extra nucleotides might be introduced at D - J_H and V_H - DJ_H junctions by the enzyme terminal deoxynucleotidyl transferase (TdT) during recombination¹⁶. This enzyme, which is present in several lymphoid lines¹⁹, is capable of catalysing polymerization of deoxyribonucleotides onto the 3'-hydroxyl terminus of an oligonucleotide primer in the absence of a template²⁰. In an attempt to correlate the expression of TdT in the 40E4-2 and 22D6-G lines with their ability to introduce extra nucleotides at the junctions of V_H , D and J_H segments, extracts prepared from these cell lines were assayed for TdT activity, as indicated by polymerization of



dGTP onto an oligo(dA) primer^{21,22}. In the presence of extract from 40E4-2 (16 µg protein), 10.0 pmol dGMP was incorporated into acid-precipitable material in 60 min at 37 °C, a level similar to that of the TdT-containing thymoma line, RLδ11. In contrast, we were unable to detect polymerization in the presence of an extract from 22D6-G cells (<0.2 pmol dGMP incorporated). Addition of extract from 22D6-G did not inhibit polymerization catalysed by the 40E4-2 extract. To confirm these results, the cell lines RLδ11, 40E4-2 and 22D6 (the parental line from which 22D6-G was cloned) were metabolically labelled with ³⁵S-methionine and extracts of these cells were assayed by immunoprecipitation with affinity-purified anti-TdT antibody. A protein of the size of TdT was precipitated from RLδ11 and 40E4-2 but not from 22D6 (Fig. 4). Also, a partial cDNA clone for TdT has been observed to hybridize to a 2.2-kb RNA species present in RLδ11 and 40E4-2 but not in 22D6 (N.L. and D.B., unpublished observations).

For five of the six V_H -to- DJ_H rearrangements that we examined, we were able to identify unambiguously in the parental cell the DJ_H segment that had participated in the rearrangement (in 40E4-2-16-2.0, V_H -to- DJ_H rearrangement resulted in deletion of the D - J_H junction). We have therefore shown directly that the DJ_H units found in these fetal liver-derived A-MuLV transformants are intermediates in V_HDJ_H assembly, as had been suggested by the results of hybridization assays reported previously^{13,14,23}. All six V_HDJ_H rearrangements characterized here have placed the V_H coding sequence out of frame with respect to the J_H coding sequence. This could reflect the small sample size or it could be that cells containing productive rearrangements are lost, perhaps through a toxic effect of free heavy chain²⁴.

We found no evidence for somatic point mutation. Comparison of the 200-base pair (bp) portion of the V_H81X sequence present in molecular clones of three V_HDJ_H recombinants (40E4-2-5-2.0, 40E4-2-16-2.0 and 22D6-G-1-2.1) with the germ-line sequence of the gene segment, and similar comparisons involving J_H segments, failed to reveal any nucleotide differences¹⁸. This is consistent with other evidence that rather than occurring coincidentally with rearrangement^{25,26} somatic point mutation occurs principally at later stages in B-lymphocyte

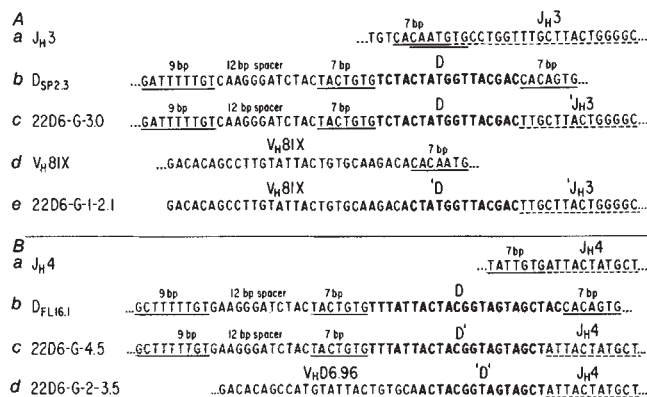


Fig. 3 V_HDJ_H assembly in the 22D6-G A-MuLV transformant. **A**, Primary and secondary rearrangement of the 3.0-kb J_H -containing *EcoRI* fragment from 22D6-G. Recombinant junctions were localized and sequence was determined as in Fig. 3. **a**, **b**, Partial nucleotide sequences of germ-line J_H3 (refs 3, 7) and $D_{SP2.3}$ (ref. 8) segments are shown. **c**, The sequence across the $D_{SP2.3}$ - J_H3 junction found in clone 22D6-G-3.0. **d**, The 3' terminal germ-line sequence of the V_H8IX gene segment¹⁸. **e**, The sequence across the recombinant junction of clone 22D6-G-2.1; this recombinant is the result of rearrangement of V_H8IX to the D - J_H3 fusion of 22D6-G-3.0. Symbols are defined in Fig. 2A. **B**, Primary and secondary rearrangement of the 4.5-kb J_H -containing *EcoRI* fragment from 22D6-G. Recombinant junctions were localized and sequence was determined as in Fig. 3. **a**, **b**, Partial nucleotide sequences of germ-line J_H4 (refs 3, 7) and $D_{FL16.1}$ (ref. 8). **c**, Sequence of the $D_{FL16.1}$ - J_H4 junction in clone 22D6-G-4.5. **d**, Partial sequence of the 22D6-G-2-3.5 recombinant, which was the product of recombination between a V_H segment and the DJ_H4 fusion of 22D6-G-4.5. Symbols are defined in Fig. 2A.

maturation that follow rearrangement. The consistent lack of somatic point mutation in rearrangements from the 40E4-2 line, which expresses TdT, implies that, contrary to a previous suggestion²⁷, the function of the enzyme in this line is not related to the process of generating somatic point mutants.

A role for TdT in the production of N regions¹⁶ is strongly implied by the observation that N regions were present at four of six independent D - J_H or V_H - DJ_H junctions in the 40E4-2 line, which expresses TdT, but none was found among the four junctions examined in the 22D6-G line that lacks the enzyme.

An alternative hypothesis, that N regions are encoded by a set of germ-line genes, seems unlikely because of their extreme heterogeneity in length and sequence. Another argument against N regions being encoded stems from the observation that the N -region sequence GTGGGGGCC is found at the junction between D_{Q52} and J_H segments in the 18-81A-2 cell line²⁸. The D_{Q52} segment is the most J_H -proximal D segment, occurring in the germ line about 700 bp to the 5' side of J_H1 . The nucleotide sequence from D_{Q52} to the J_H cluster has been determined previously⁵; this interval does not contain the 9-bp N region sequence of 18-81A-2. Thus, unless we invoke two acts of inversional recombination, so as to transpose a sequence originally 5' to a site between D_{Q52} and J_H , we must conclude that the N

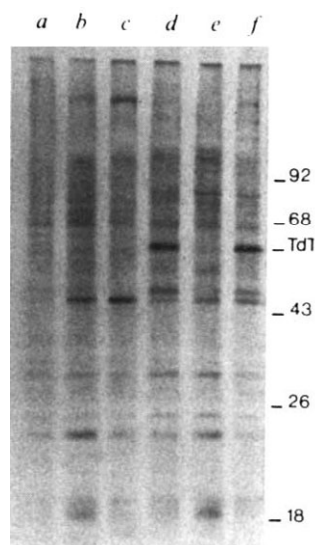


Fig. 4 Assay for terminal deoxynucleotidyl transferase by immunoprecipitation. TdT was assayed in metabolically labelled extracts from cell lines RL811 (lanes **a**, **d**), 22DG (lanes **b**, **e**) and 40E4-2 (lanes **c**, **f**), as described below. (Cell line 22D6 is the parent line from which clone 22D6-G was derived.) Immunoprecipitations were performed with normal rabbit serum (lanes **a**-**c**) or with rabbit anti-TdT antiserum (lanes **d**-**f**). The positions of molecular weight standards and their sizes ($\times 10^{-3}$) are indicated.

Methods: Cells (4×10^6) were labelled for 5 h at 37 °C in 2 ml methionine-free RPMI-1640 supplemented with 10% dialysed, inactivated fetal calf serum and 500 μ Ci 35 S-methionine. Cells were washed in PBS and lysed by addition of 1 ml RIPA buffer³⁴ containing 0.1 mM phenylmethylsulphonyl fluoride. Samples, containing 10^7 c.p.m. 35 S-labelled protein, were incubated with 5 μ l normal rabbit serum for 1 h at 4 °C. Protein A-containing *Staphylococcus aureus* (25 μ l of a 10% suspension) was added and incubation was continued for 30 min. Samples were cleared by centrifugation at 12,000g for 2 min. Precleared samples were incubated with 5 μ l rabbit antiserum against calf TdT³⁵ or with 5 μ l normal rabbit serum for 2 h at 4 °C. Protein A-containing *S. aureus* (25 μ l of a 10% suspension) was added and incubation was continued for 30 min. RIPA buffer (1 ml) was added and samples were spun for 15 s at 12,000g. Pellets were washed twice with RIPA buffer. Immunoprecipitated protein was analysed by SDS-polyacrylamide gel electrophoresis and autoradiography.

region present at this D_{Q52} - J_H junction was not represented in the germ line. N regions are therefore likely to be synthesized *de novo* and TdT is likely the responsible enzyme.

We thank Keith Blackwell for advice and assistance in the isolation of the 22D6 rearrangements. S.V.D. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research. G.D.Y. is supported by NIH training grant AI-20047. F.A. is an Irma T. Herschel Career Scientist. This work was supported by ACS grant NP-393, NIH grant AI-20047 and a Searle Foundation award to F.A. and ACS grant IM-355 to D.B.

Received 25 May; accepted 19 September 1984.

1. Tonegawa, S. *Nature* **302**, 575-581 (1983).
2. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19**, 981-992 (1980).
3. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676-683 (1980).
4. Bernard, O. & Gough, N. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3630-3634 (1980).
5. Sakano, H., Kurosawa, Y., Weigert, M. & Tonegawa, S. *Nature* **290**, 562-565 (1981).
6. Kurosawa, Y. *et al.* *Nature* **290**, 565-570 (1981).
7. Gough, N. M. & Bernard, O. *Proc. natn. Acad. Sci. U.S.A.* **78**, 509-513 (1981).
8. Kurosawa, Y. & Tonegawa, S. *J. exp. Med.* **155**, 201-218 (1982).
9. Rosenberg, N. & Baltimore, D. *J. exp. Med.* **143**, 1453-1463 (1976).
10. Waneck, G. L. & Rosenberg, N. *Cell* **26**, 79-89 (1981).
11. Siden, E. J., Baltimore, D., Clark, D. & Rosenberg, N. E. *Cell* **16**, 389-396 (1979).
12. Alt, F., Rosenberg, N., Lewis, S., Thomas, E. & Baltimore, D. *Cell* **27**, 381-390 (1981).
13. Sugiyama, H. *et al.* *Nature* **303**, 812-815 (1983).
14. Alt, F. *et al.* *EMBO J.* **3**, 1209-1219.
15. Goff, S., Gilboa, E., Witte, O. N. & Baltimore, D. *Cell* **22**, 777-785 (1980).
16. Alt, F. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4118-4122 (1982).
17. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. *Sequences of Proteins of Immunological Interest* (U.S. Department of Health and Human Services, NIH, Bethesda, 1983).

18. Yancopoulos, G. D. *et al.* *Nature* **311**, 727-733 (1984).
19. Silverstone, A. E. *et al.* *Cold Spring Harb. Conf. Cell Proliferation* **5**, BA, 433-453 (1978).
20. Bollum, F. J. *Enzymes* **10**, 145-171 (1974).
21. Kung, P. C., Silverstone, A. E., McCaffrey, R. & Baltimore, D. *J. exp. Med.* **141**, 855-865 (1975).
22. McCaffrey, R. P., Smoler, D. F. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **70**, 521-525 (1973).
23. Yaoita, Y. *et al.* *Nucleic Acids Res.* **11**, 7303-7316 (1983).
24. Kohler, G. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2197-2199 (1980).
25. Gearhart, P. J., Johnson, N. D., Douglas, R. & Hood, L. *Nature* **291**, 29-34 (1981).
26. Bothwell, A. L. M. *et al.* *Cell* **24**, 625-637 (1981).
27. Baltimore, D. *Nature* **248**, 409-411 (1974).
28. Alt, F., Rosenberg, N., Enea, V., Siden, E. & Baltimore, D. *Molec. Cell Biol.* **2**, 386-400 (1982).
29. Alt, F. W., Enea, V., Bothwell, A. L. M. & Baltimore, D. *Cell* **21**, 1-12 (1980).
30. Blattner, F. R. *et al.* *Science* **196**, 161-169 (1977).
31. Sternberg, N. T., Tiemier, D. & Enquist, L. *Gene* **1**, 255-280 (1977).
32. Benton, W. D. & Davis, R. W. *Science* **196**, 180-182 (1977).
33. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
34. Gilead, Z. *et al.* *Nature* **264**, 263-266 (1976).
35. Silverstone, A., Sun, L., Witte, O. N. & Baltimore, D. *J. biol. Chem.* **255**, 791-796 (1980).

A rhodopsin is the functional photoreceptor for phototaxis in the unicellular eukaryote *Chlamydomonas*

Kenneth W. Foster*‡§, Jureepan Saranak*‡, Nayana Patel*, Gerald Zarilli†, Masami Okabe†, Toni Kline* & Koji Nakanishi†§

* Department of Pharmacology, Mt Sinai School of Medicine, City University of New York, New York, New York 10029, USA

† Department of Chemistry, Columbia University, New York, New York 10027, USA

Rhodopsin is a visual pigment ubiquitous in multicellular animals. If visual pigments have a common ancient origin, as is believed, then some unicellular organisms might also use a rhodopsin photoreceptor^{1,2}. We show here that the unicellular alga *Chlamydomonas* does indeed use a rhodopsin photoreceptor. We incorporated analogues of its retinal chromophore into a blind mutant; normal photobehaviour was restored and the colour of maximum sensitivity was shifted in a manner consistent with the nature of the retinal analogue added. The data suggest that 11-*cis*-retinal is the natural chromophore and that the protein environment of this retinal is similar to that found in bovine rhodopsin, suggesting homology with the rhodopsins of higher organisms. This is the first demonstration of a rhodopsin photoreceptor in an alga or eukaryotic protist and also the first report of behavioural spectral shifts caused by exogenous synthetic retinals in a eukaryote. A survey of the morphology and action spectra of other protists suggests that rhodopsins may be common photoreceptors of chlorophycean, prasinophycean and dinophycean algae. Thus, *Chlamydomonas* represents a useful new model for studying photoreceptor cells.

Chlamydomonas is an aquatic organism of 10 µm diameter with a single eye and two flagella³ which together guide the swimming cell in its light environment. A coloured quarter-wave-layered eyespot focuses incoming light onto an overlying patch of plasma membrane containing ~10⁵ receptor pigment molecules and also prevents light from passing through the cell to the receptor pigment². As the cell rotates while swimming, its eye conically scans a pattern of light which modulates conductance changes that control the steering of its flagella, thus forming a simple, but nevertheless complete visual system. All the essential elements of the photoreceptor cells of a multicellular animal are present².

Although there are many light-sensitive pigments, particularly in protists⁴, the receptor pigments in the eyes of all multicellular animals studied so far contain 11-*cis*-retinal as the chromophore; in addition, some freshwater fish and amphibians use 11-*cis*-3,4-dehydroretinal as a second chromophore. We used the following rationale to check for the presence of a retinal chromophore in *Chlamydomonas*. If a retinal moiety were the chromophore, replacing it by other retinal analogues would shift the action spectrum (the relative sensitivity of the cells to different wavelengths of light) compared with the natural pigment; such a shift in action spectrum would prove the incorporation and function of retinal. Nonspecific binding of retinals to protein would not produce the anticipated shifts. The behaviour used to test the photoreceptor response was that of phototaxis^{2,5}—the swimming of an aquatic cell towards or away from light.

Numerous retinal analogues have been bound to bovine opsin and bacteriorhodopsin *in vitro*⁶⁻⁹ to give pigments absorbing at different wavelengths of light. At least four analogues have recently been shown to shift the behavioural action spectrum of *Halobacterium*^{10,11} and a similar rationale using a riboflavin analogue was applied to determine the nature of the chromophore for phototropism in the fungus *Phycomyces*¹².

As with the *Phycomyces* studies in which a riboflavin auxotroph was used¹², we used a blind mutant incapable of synthesizing retinal in the dark to measure incorporation of analogues. As any mutant blocking carotenoid synthesis would also block retinal synthesis, we surveyed all carotenoid mutants we could obtain; as hypothesized, these are blind compared with wild type. We chose the mutant FN68 (Car-1) because of the comparative slowness of its light induction of carotenoids (K.W.F. *et al.*, in preparation) and its 1,000–10,000-fold increased threshold sensitivities relative to wild type (compare Fig. 1B, C with D). The carotenoid biosynthesis of the mutant¹³ is blocked in the dark at a step before phytoene. The mutant is negatively phototactic, that is, it normally swims away from light. The wild type (for carotenogenesis) (Car⁺ agg⁻ cell, designated FN13) was a negatively phototactic strain (derived from culture 806 of *Chlamydomonas reinhardtii* 137c(-); given by Robert Smyth).

We used threshold measurements of phototaxis (Fig. 1) to determine the sensitivity of the cells to a particular colour of light. The sensitivity or reciprocal of the threshold was plotted as a function of wavenumber (wavelength⁻¹) to give a phototaxis action spectrum for each analogue tested (Fig. 2A, a–g). Peaks were determined by fitting the data to a standard curve¹⁴. As in other animals, *trans*-retinol (vitamin A) and β-carotene function as well as retinal, while retinoic acid has no effect.

To provide a standard to determine the effect of the protein environment on a retinal analogue, a simple reference environment was needed. As it is known that the retinal in other rhodopsins is attached to the protein as a protonated Schiff base (SBH⁺), the absorption of the analogue in that form, attached to a small compound such as *n*-butylamine in the solvent methanol, provides a useful reference. The difference (in wavenumber) between the absorption maximum of SBH⁺ in methanol and those of the pigments has been defined as the opsin shift¹⁵. The action spectra maxima for *Chlamydomonas* are not only shifted towards the red away from the reference maxima provided by the protonated Schiff bases (SBH⁺), but the trends of these opsin shifts (Fig. 3) are very similar to those encountered for bovine rhodopsins. The opsin shifts from the SBH⁺ model vary over a narrow range, with the 9-*cis* analogues having a smaller opsin shift than the 11-*cis* analogues. In contrast, the shifts observed here are markedly different from those encountered with bacteriorhodopsin, which, unlike visual pigments, absorb at 570 nm and only accept *trans*- and 13-*cis*-retinals¹⁶. The natural or light-induced action spectra maximum for *Chlamydomonas* is at 503 nm, whereas the maxima resulting from *trans*- and 11-*cis*-retinal incorporation are at 505 and 501 nm, respectively (Fig. 2). As these values are within experimental error, it is likely that the retinals are isomerized in the dark (to 11-*cis* by an isomerase?). However, the maximum at 488 nm caused by incorporation of 9-*cis* is outside the range of error and parallels the 485 nm maximum of bovine 9-*cis* rhodopsin. These findings suggest that *Chlamydomonas*, a chlorophycean, has a pigment homologous to bovine rhodopsin. We suspect the existence of rhodopsins in other algae also (Fig. 4). These pigments show a variation in their maximum absorption wavelength, from 457 to 493 nm, which implies a similar variety of chromophore environments to those in multicellular animals. Although they are probably all rhodopsin-like pigments, the nature of the chromophores in the Prasinophyceae and Dinophyceae remains to be proven.

Our results involving incorporation of retinal analogues have established *Chlamydomonas* as a viable model for studies of rhodopsin photoreceptors. A major advantage of this system is that it is already widely used for the study of basal bodies¹⁷, circadian rhythms^{18,19} and flagellar architecture and function^{20,21,38}, among others. Furthermore, the visual pigment and ionic processing elements are readily accessible as they lie in the plasma membrane at the surface of the cell. The versatility of this genetically well-studied microorganism for the study of photoreception, together with the apparent close homology of its photoreceptor cells to those of multicellular organisms, make it an excellent model. Use of this simple unicellular system

‡ Present address: Physics Department, 201 Physics Building, Syracuse University, Syracuse, New York 13210, USA.

§ To whom reprint requests should be addressed.

Fig. 1 A, The experimental set-up at time 0 and at 600 s. B–D, Dose-response curves, using 540 nm light with: B, FN13 cells (normal for carotenoids); C, FN68 cells and added 11-*cis*-retinal (25 μ M); and D, FN68 cells without any added retinal.

Methods: FN68 cells were taken from slant cultures and grown on plates (salt, acetate Bacto-tryptone media with no yeast)²³. These cells were transferred to HSM liquid (9.3 mM NH_4Cl , 81 μM MgSO_4 , 0.1 mM CaCl_2 , 8.27 mM K_2HPO_4 , 5.3 mM KH_2PO_4 , 15 mM Na acetate, plus trace elements²⁴) and grown for 5–7 days in the dark at 18 °C. These vegetative cells were converted to gametes by nitrogen starvation in NMM (81 μM MgSO_4 , 0.1 mM CaCl_2 , 4.13 mM K_2HPO_4 , 2.65 mM KH_2PO_4 , plus trace elements²⁴). On the day before the experiment, vegetative cells were centrifuged out at 1,500 r.p.m. for 3 min. The pellets were washed two or three times with NMM and resuspended in NMM to a concentration of $5\text{--}7 \times 10^6$ cells ml^{-1} . To the final NMM and cell suspension 25 μM of each retinal analogue and 0.025% α -tocopherol (as an antioxidant) were added. There was a dose-related effect of the analogues, and 25 μM was the lowest concentration that resulted in nearly full recovery with the most effective analogues. All analogues were transferred to methanol solvent immediately before addition to the cells. α -Tocopherol was also dissolved in methanol. The final concentration of methanol in the cell suspension was always <0.5%. Cells were left overnight for gametes to develop and incorporate the analogues. All cells were checked for motility and bacterial contamination. 4–5 ml of the cell suspension were poured into 54 mm diameter polystyrene Petri plates and placed at various distances from a stabilized quartz-iodide light source, the colour of which was selected with a series of interference filters. As the same set of filters was used for each analogue, the error in the comparative data is very small. To ensure a high degree of accuracy, two interference filters in series were used on the rapid fall-off in response to the red. Each light condition was measured with a 400 series Photodyne radiometer calibrated to $\pm 5\%$ for each wavelength. The energies were converted to absolute irradiances and corrected for the small amount of plate absorption. To measure action spectra, dose-response curves of phototactic rate versus light intensity were obtained. The distance travelled by the trailing edge of phototactic cells was measured for a 600-s period. Any effects of light scattering were minimized as light passed through the clear region created by cells swimming away from the light. The amount of opsin available may differ from day to day and the absolute sensitivity at all wavelengths may be changed; therefore, a complete action spectrum for one analogue was determined for one set of cells in one day. A control (undoped cells) was always included and measured for phototactic threshold. The procedure was repeated on a second or third day, if necessary, and all data points plotted with a common symbol for each day's set of data, as shown in Fig. 2. Unfortunately, in our mutant some induction of carotenoid and retinal synthesis (K.W.F. *et al.*, in preparation) occurs in the dark in the presence of retinals and retinal oxidation products (as also found in *Phycomyces*)²⁵. However, we minimized oxidation by limiting exposure to light and oxygen and we added the analogues in optimum amounts; thus chemical induction did not interfere with our experiments. The slopes of the dose-response curves vary with wavelength as the conical scanning mechanism (see ref. 2 for details) results in a signal proportional to the magnitude of contrast modulation, which varies with wavelength. Therefore, we used the threshold for phototaxis rather than the intensity to give a standard response. Using this method, the presence of low concentrations of analogues, which contribute <0.001 absorbance units to the contrast modulation, has no effect on the action spectra. For threshold, we used the linear extrapolation to zero response, on a linear plot of phototactic rate as a function of the log of light intensity.

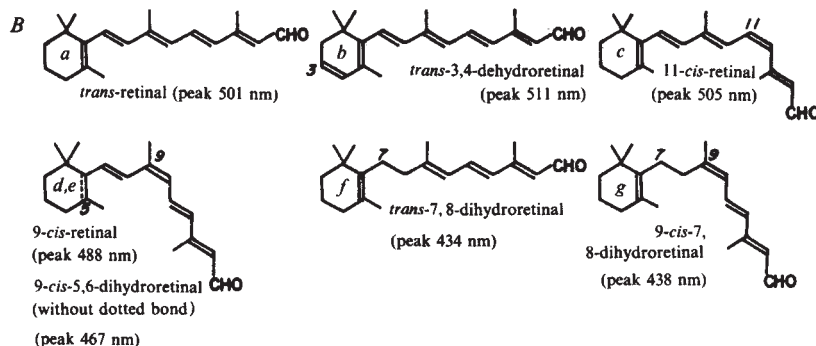
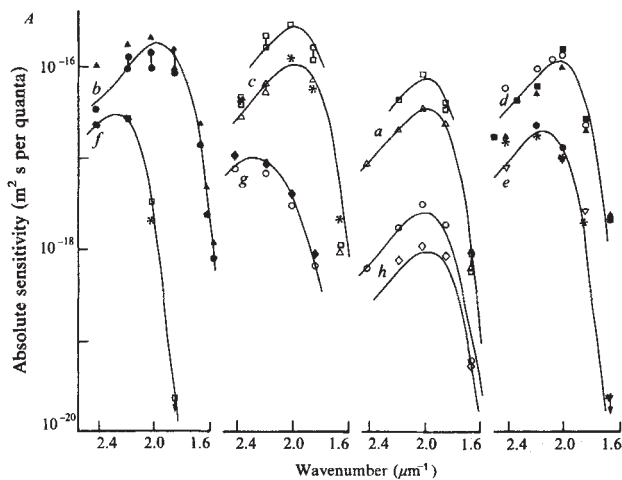
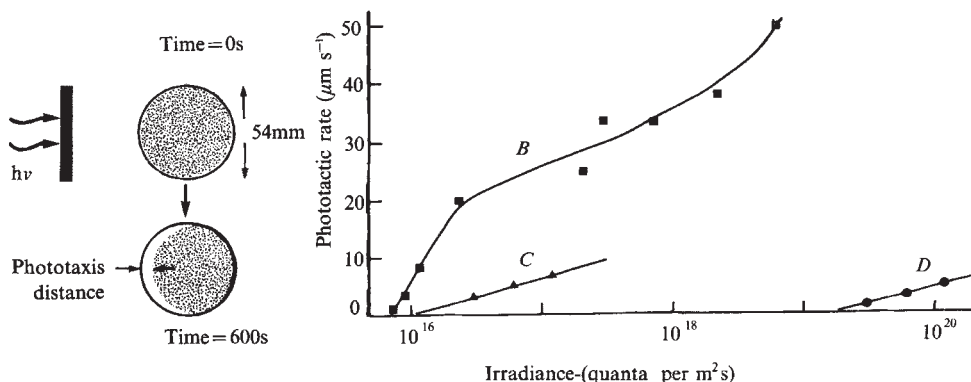


Fig. 2 A, The action spectra for different incorporated analogues. B, The structure and excitation maxima of the analogues: *a*, *trans*-retinal (peak 501 ± 5 nm); *b*, *trans*-3,4-dehydroretinal²⁶ (peak 511 ± 2 nm); *c*, 11-*cis*-retinal (peak 505 ± 6 nm); *d*, 9-*cis*-retinal (with dotted bond) (peak 488 ± 5 nm); *e*, 9-*cis*-5,6-dihydroretinal²⁷ (without dotted bond) (peak 467 ± 5 nm); *f*, *trans*-7,8-dihydroretinal²⁷ (peak 434 ± 3 nm); *g*, 9-*cis*-7,8-dihydroretinal²⁷ (peak 438 ± 5 nm); and *h*, light induced at 540 nm for 1.8×10^3 s at an irradiance of 4.2×10^{19} quanta m^{-2} per s (upper curve) and 2.5×10^{19} quanta m^{-2} per s (lower curve) (peak 503 ± 2 nm).

Methods: The action spectra were determined by the method described in Fig. 1 legend. log of sensitivity (reciprocal of threshold) on an absolute scale is plotted on the ordinate as a function of the wavenumber (wavelength⁻¹) (μm^{-1} , 10,000 cm^{-1}); when plotted in this way, the shape of the curve of rhodopsin excitation does not change despite shifts of maxima. The ordinate is given an absolute scale to compare the sensitivities of each analogue added at the same concentration. The absolute sensitivity of an analogue is a function of its affinity and quantum efficiency; we did not distinguish between these two parameters in this study. In some cases a second partial curve is drawn through a particular day's data because on that day the analogue's own absolute sensitivity was different. The data were fitted to a standard rhodopsin curve¹⁴ (solid lines) and from this fit the peak wavelengths were obtained. The one exception is 9-*cis*-7,8-dihydroretinal (*g*) for which the curve is distorted by competition with the induced native chromophore (K.W.F. *et al.*, in preparation). To obtain the best estimate of the maxima of the data curve, however, a 1/6 component of natural chromophore was subtracted and the standard curve fitted to the result. In all other cases we are confident of complete dominance of the substitution.

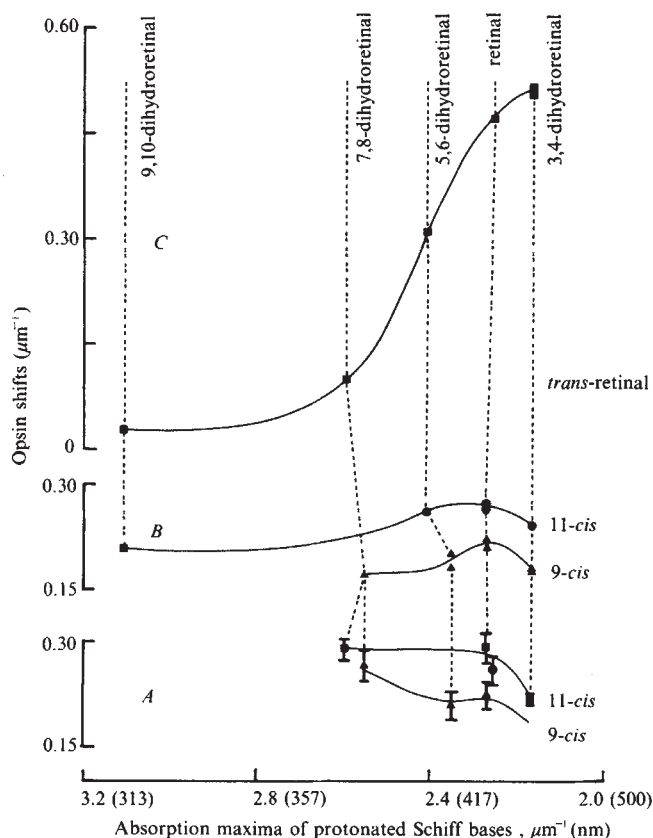


Fig. 3 Chromophore environments of *Chlamydomonas* rhodopsin (A), bovine rhodopsin (B) and bacteriorhodopsin (C). Retinal chromophores in rhodopsins are bound to opsin via a protonated Schiff base linkage, absorption maxima being considerably redshifted from those (in methanol) of protonated Schiff bases formed with *n*-butylamine (SBH⁺). This redshift, reflecting the unique rhodopsin protein environment of retinal, expressed in wavenumbers (μm^{-1} , $10,000\text{ cm}^{-1}$), is defined as the opsin shift¹⁵. Retinals nonspecifically bound to proteins in the SBH⁺ form absorb at the SBH⁺ wavelength²⁸, and in free solution in the UV. In *Chlamydomonas* the action spectra are again redshifted from the SBH⁺ form and are fitted so well to the standard rhodopsin curve, so nonspecific protein attachment of retinals has no effect on phototaxis. For *Chlamydomonas* rhodopsin we define opsin shift to include the difference between the maxima of SBH⁺ and those of its action spectra. An analogue added *trans* will probably be isomerized by the cell to 11-*cis*, but the SBH⁺ values are nearly identical. Opsin shifts of the excitation maxima of *Chlamydomonas* phototaxis (A) and of absorption maxima of bovine rhodopsins (B) and bacteriorhodopsin (C) resulting from incorporation of various analogues are plotted against maxima of the corresponding SBH⁺, expressed in μm^{-1} . Data points in the 9-*cis* ($\blacktriangle$), 11-*cis* ($\bullet$) and *trans* ($\blacksquare$) series are connected laterally for clarity. The vertical dashed lines interconnect the double bond isomers of respective retinals. The values for the excitation maxima of *Chlamydomonas* are listed in Fig. 2 legend. The values for absorption maxima of SBH⁺, bovine rhodopsins (RH) and bacteriorhodopsins (BR) are as follows: *trans*-retinal (a of Fig. 2B), SBH⁺ 443 nm (ref. 29), BR 560 nm (ref. 15); *trans*-3,4-dehydroretinal (b), SBH⁺ 460 nm (ref. 28), BR 603 nm (ref. 30) or 599 nm (ref. 31); 11-*cis*-3,4-dehydroretinal, SBH⁺ 460 nm (assumed to be like *trans* as only a few nanometres different)²⁸, RH 517 nm (ref. 28); 9-*cis*-3,4-dehydroretinal, SBH⁺ 460 nm (assumed to be like *trans*), RH 501 nm (ref. 28) or 500 nm (ref. 32); 11-*cis*-retinal (c), SBH⁺ 440 nm (ref. 15), RH 498 nm (ref. 28) or 500 nm (ref. 15); 9-*cis*-retinal (d), SBH⁺ 440 nm (ref. 15), RH 485 nm (ref. 15) or 487 nm (ref. 28); *trans*-5,6-dihydroretinal, SBH⁺ 415 nm (ref. 28), BR 476 nm (ref. 15); 11-*cis*-5,6-dihydroretinal, SBH⁺ 415 nm (assumed to be like *trans*), RH 465 nm (ref. 28); 9-*cis*-5,6-dihydroretinal (e), SBH⁺ 425 nm (ref. 15), RH 465 nm (ref. 28) or 460 nm (ref. 15); *trans*-7,8-dihydroretinal (f), SBH⁺ 385 nm (ref. 15), BR 400 nm (ref. 15); 9-*cis*-7,8-dihydroretinal (g), SBH⁺ 392 nm (ref. 33), RH 420 nm (ref. 15); and *trans*-9,10-dihydroretinal, SBH⁺ 322 nm (ref. 15), RH 345 nm (ref. 15), BR 325 nm (ref. 15).

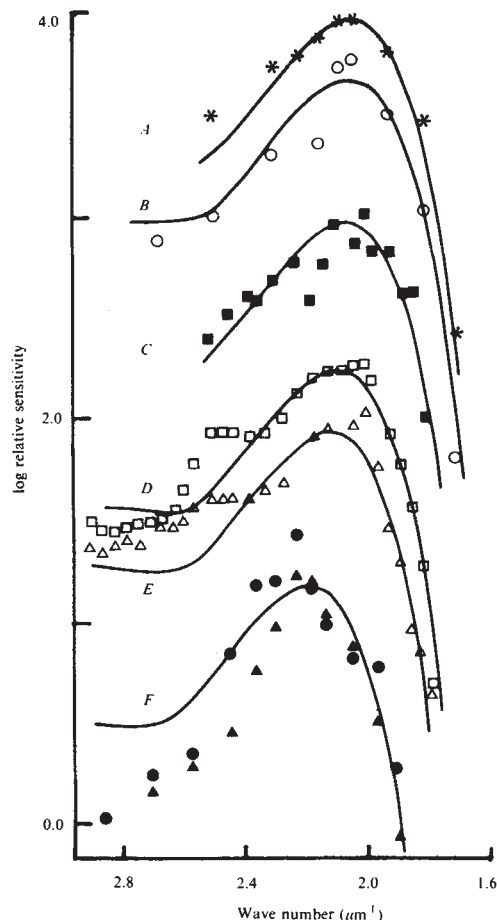


Fig. 4 Action spectra of algae taken from the literature. The most probable homologous characteristic to identify between alga eyes and those of multicellular organisms is an oriented rhodopsin-like protein formed in the plasma membrane regulating ion currents. The use of a photoreceptor at the quarter-wave position overlying a quarter-wave stack antenna in several algal classes (see ref. 2 for details) requires an oriented receptor for optimal functioning. On the grounds of morphology and action spectra, therefore, we have redrawn several action spectra from the literature: log of sensitivity (reciprocal of threshold or quantum efficiency) is plotted as a function of the wavenumber (wavelength⁻¹) (μm^{-1} , $10,000\text{ cm}^{-1}$) as in Fig. 2A. To facilitate comparison, the data have been arbitrarily displaced on the ordinate and the same standard curve¹⁴ has been horizontally shifted to best fit the data from the maximum to the red cut-off of sensitivity. We anticipate significant distortion in the action spectra above $2.3\text{ }\mu\text{m}^{-1}$ (to the violet side of 430 nm) due to activation of photosynthesis. A, The 'stop' response ($\ast$) of *Volvox aureus* (a chlorophycean) (peak 493 nm)³⁴; B, the threshold positive phototaxis response ($\circ$) of *V. aureus* (peak 490 nm)³⁴; C, the photoreceptor electrical potential ($\blacksquare$) of *Haematococcus pluvialis* (a chlorophycean) (peak 483 nm)³⁵; D, the threshold negative phototaxis response ($\square$) of *Platymonas subcordiformis* (a prasinophycean) (peak 478 nm)³⁶; E, the threshold positive phototaxis response ($\triangle$) of *Pl. subcordiformis* (peak 472 nm)³⁶; F, the stop response ($\blacktriangle$) and positive phototaxis (recalculated) ($\bullet$) of *Gymnodinium splendens* (a dinophycean) (peak 457 nm)³⁷.

should allow us to elucidate the active sites of protein interaction; transduction aspects; adaptation; signal processing; membrane, receptor and chromophore biosynthesis and maintenance; nutritional effects on the lipid environment of the receptor; and effects of cell homeostasis.

The use of eukaryotic rhodopsins as visual receptors may indeed be quite ancient and as common in several classes of algae as in higher animals. The use of molecular biology²² to check for homology between the rhodopsins found throughout these algae and higher animals should prove exciting.

We thank Dr Robert D. Smyth for discussions and the impor-

tant suggestion that a rhodopsin might be the photoreceptor in *Chlamydomonas*. We also thank the NIH for support (grant EY 03760 to K.W.F. and N.P. and grant EY 01253 to K.N.) and

the many who lent us equipment to make these experiments possible. W.-Y. Wang (University of Iowa) supplied the FN68 mutant.

Received 22 February; accepted 11 July 1984.

1. Foster, K. W. & Smyth, R. D. *Fedn Proc.* **39**, 2139 (1980).
2. Foster, K. W. & Smyth, R. D. *Microbiol. Rev.* **44**, 572-630 (1980).
3. Ehrenberg, C. G. *Die Infusionstierchen als Vollkommene Organismen*, Leipzig (1838).
4. Song, P.-S. *Photochem. Photobiol. Rev.* **7**, 77-140 (1983).
5. Greuet, C. *Année biol.* **21**, 97-141 (1982).
6. Balogh-Nair, V. & Nakanishi, K. in *New Comprehensive Biochemistry* Vol. 3 (ed. Tamm, Ch.) (Elsevier, Amsterdam, 1982).
7. Balogh-Nair, V. & Nakanishi, K. *Meth. Enzym.* **88**, 496-506 (1982).
8. Kropf, A., Whittenberger, B. P., Goff, S. P. & Waggoner, A. S. *Expl Eye Res.* **17**, 591-606 (1973).
9. Motto, M. G., Sheves, M., Tsujimoto, K., Balogh-Nair, V. & Nakanishi, K. *J. Am. chem. Soc.* **102**, 7947-7949 (1980).
10. Schimz, A., Sperling, W., Ermann, P., Bestmann, H. J. & Hildebrand, E. *Photochem. Photobiol.* **38**, 417-423 (1983).
11. Schimz, A., Sperling, W., Hildebrand, E. & Kohler-Hahn, D. *Photochem. Photobiol.* **36**, 193-196 (1982).
12. Otto, M. K., Jayaram, M., Hamilton, R. M. & Deibrock, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 266-269 (1981).
13. Wang, W.-Y. *Genetics* **91**, s134 (1979).
14. Knowles, A. & Dartnall, H. J. in *The Eye* Vol. 2B (ed. Davson, H.) 76 (Academic, New York, 1977).
15. Nakanishi, K., Balogh-Nair, V., Arnaboldi, M., Tsujimoto, K. & Honig, B. *J. Am. chem. Soc.* **102**, 7945-7947 (1980).
16. Oesterheld, D. & Stoekenius, W. *Nature new Biol.* **233**, 149-152 (1971).

17. Huang, B., Ramanis, Z., Dutcher, S. K. & Luck, D. J. C. *Cell* **29**, 745-753 (1982).
18. Straley, S. C. & Bruce, V. G. *Pl. Physiol.* **63**, 1175-1181 (1979).
19. Goodenough, J. E., Bruce, V. G. & Carter, A. *Biol. Bull. mar. biol. Lab., Woods Hole* **161**, 371-381 (1981).
20. Hoops, H. J. & Witman, G. B. *J. Cell Biol.* **97**, 902-908 (1983).
21. Brokaw, C. J. & Luck, D. J. C. *Cell Motility* **4**, 131-150 (1983).
22. Nathans, J. & Hogness, D. S. *Cell* **34**, 807-814 (1983).
23. Nicholson-Guthrie, C. S. & Hudock, G. A. *J. gen. Microbiol.* **129**, 159-165 (1983).
24. Hutner, S. H., Provosoli, L., Schatz, A. & Haskins, C. P. *Proc. Am. phil. Soc.* **94**, 152-170 (1950).
25. Eslava, A. P., Alvarez, M. I. & Cerdá-Olmedo, E. *Eur. J. Biochem.* **48**, 617-623 (1974).
26. Hubbard, R., Brown, P. K. & Bownds, D. *Meth. Enzym.* **33C**, 615-653 (1971).
27. Arnaboldi, M., Motto, M. G., Tsujimoto, K., Balogh-Nair, V. & Nakanishi, K. *J. Am. chem. Soc.* **101**, 7082-7086 (1979).
28. Blatz, P. E., Dewhurst, P. B., Balasubramanian, V., Balasubramanian, P. & Lin, M. *Photochem. Photobiol.* **11**, 1-15 (1970).
29. Erickson, J. O. & Blatz, P. E. *Vision Res.* **8**, 1367-1375 (1968).
30. Tokunaga, F. & Ebrey, T. G. *Biochemistry* **17**, 1915-1922 (1978).
31. Sperling, W. & Schimz, A. *Biophys. Struct. Mech.* **6**, 165-169 (1980).
32. Azuma, M., Azuma, K. & Keto, Y. *Biochim. biophys. Acta* **295**, 520-527 (1973).
33. Honig, B. et al. *J. Am. chem. Soc.* **101**, 7084-7086 (1979).
34. Schletz, K. Z. *Pflanzenphysiol.* **77**, 189-211 (1976).
35. Litvin, F. F., Sineschekov, O. A. & Sineschekov, V. A. *Nature* **271**, 476-478 (1978).
36. Halldal, P. *Physiologia Pl.* **14**, 133-139 (1961).
37. Forward, R. B. Jr *J. Protozool.* **21**, 312-315 (1974).
38. Lack, D. J. L. *J. Cell Biol.* **98**, 789-794 (1984).

Co-localization of neurotensin-like immunoreactivity and ³H-glycine uptake system in sustained amacrine cells of turtle retina

Reto Weiler* & Alexander K. Ball

Department of Anatomy and Lion's Sight Centre, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 4N1

Amacrine cells are axonless intrinsic neurones of the vertebrate retina which have cell bodies in the proximal inner nuclear layer and processes contributing to the synaptic network of the inner plexiform layer. They receive input from bipolar, interplexiform and other amacrine cells, and synapse onto these and ganglion cells^{1,2}. Amino acid and monoamine transmitters are found in most retinal neurones³, but peptide transmitters are exclusively located in amacrine cells⁴. Only one neuropeptide, amino acid or monoamine transmitter exists in any single amacrine cell population^{4,5}. Coexistence of neuropeptides with classical transmitters has been demonstrated histologically in many neurones of the central nervous system⁶, but the physiological relevance of these findings is unknown⁷. We report here evidence of such coexistence in retinal amacrine cells of the turtle, *Pseudemys scripta elegans*. Using combined immunocytochemistry and autoradiography, we have localized both neurotensin-like immunoreactivity and a high affinity uptake system for ³H-glycine to the same amacrine cell, implying that this cell type may use both substances as neurotransmitters. We also present electrophysiological evidence that this type of amacrine cell responds to photic stimulation with a sustained and graded membrane depolarization.

We examined formalin-fixed frozen sections of turtle retina by the peroxidase-antiperoxidase method⁸ and found neurotensin-like immunoreactivity in four different amacrine cell types (Fig. 1a-d)^{9,10}. Two of these types occurred more frequently (Fig. 1a, b) than the other two (Fig. 1c, d). Type A cells (Fig. 1a) are large, with the soma located in the first row of amacrine cells. They send a single primary process to the middle of the inner plexiform layer before branching in the proximal half of this layer. Type B cells (Fig. 1b) are smaller, with the soma located in the first or second row of amacrine cells. They stain much more intensely than type A cells. Beaded processes from type B cells extend along the inner nuclear-plexiform layer border for a short distance before dropping to the proximal half

of the inner plexiform layer. Both cell types are identical to other neurotensin-like immunoreactive cells described recently¹¹. Less frequent neurotensin-like immunoreactive cell types were found in the distal half of the inner plexiform layer (large pyramidal soma, Fig. 1c) and the ganglion cell layer, where a thick primary process extended into the middle of the inner plexiform layer before branching (Fig. 1d).

Somas of varying sizes in different rows of the inner nuclear layer accumulated ³H-glycine (Fig. 1e). Labelling of the inner plexiform layer was diffuse, with no evident banding pattern. Some of the somas which accumulated ³H-glycine also showed neurotensin-like immunoreactivity. We identified these somas as type B cells on the basis of their shape, location and the distribution of their processes. (Somas of type A cells are characterized by their single process, which does not branch until it reaches the proximal inner plexiform layer, whereas type B somas are characterized by several processes which branch immediately after leaving the soma; Fig. 1a, b.) The diffuse labelling pattern of ³H-glycine uptake within the inner plexiform layer corresponds well with the branching pattern of type B cells. In thin sections (0.5 µm), the co-localization of the two markers in a type B soma was readily identifiable (Fig. 1f). Such preparations also confirmed that ³H-glycine uptake and neurotensin-like immunoreactivity was co-localized in type B but not in type A cells (Fig. 1f). Although type B cells all accumulate ³H-glycine, they make up only 7% of the ³H-glycine-accumulating cells in the turtle retina. The co-localization of these two markers in one cell implies that this neurone uses both an amino acid and a neuropeptide as neurotransmitters. We also investigated the possible co-localization of neurotensin-like immunoreactivity with [³H]-γ-aminobutyric acid and dopamine accumulation, but evidence of such coexistence could not be established.

In parallel experiments we used intracellular recording and staining techniques to examine the function of type B cells. The processes of most injected amacrine cells (n = 47) were either mono- or multi-stratified within the inner plexiform layer, covering 10-70% of it in transverse sections. This branching pattern differed in cells of only one group (n = 6), which had somas located in the proximal inner nuclear layer. A few processes from cells of this smaller group left the soma close to the inner nuclear-plexiform layer border, descending through the total inner plexiform layer and branching at different levels with an increasing density of fine branches in the proximal half (Fig. 2a). The six injected cells of this type were morphologically homogeneous, differing from one another only in the size of their receptive field. The size and location of the soma of these cells, and the diffuse pattern of their process distribution within

* Present address: Zoological Institute, University of Munich, Luisenstrasse 14, 8 Munich 2, FRG.

Fig. 1 *a*, Neurotensin (NT)-like immunoreactivity in a large amacrine cell of the turtle retina (A). A single primary process distributes fibres to the proximal half of the inner plexiform layer (ipl). Neurotensin-like immunoreactivity was localized using primary rabbit antiserum against neurotensin (dilution 1:1,000), goat anti-rabbit γ -globulin (Sigma) (dilution 1:20) and rabbit peroxidase-antiperoxidase complex (Miles) (dilution 1:50). Background staining was reduced with the addition of 1% normal goat serum (Sigma) to all reagents. *b*, Neurotensin-like immunoreactivity in a small amacrine cell of the turtle retina (B). Numerous processes distribute fibres to the proximal half of the inner plexiform layer. *c*, Neurotensin-like immunoreactivity in an interstitial cell of the turtle retina. The soma occupies the distal half of the inner plexiform layer and processes ramify in its proximal half. *d*, Neurotensin-like immunoreactivity in a displaced amacrine cell. A single primary process distributes fibres to the proximal half of the inner plexiform layer. *e*, Autoradiograph showing ^3H -glycine accumulation by several turtle amacrine cells (arrow-heads). Labelling of the inner plexiform layer is diffuse. The section was overexposed (2 weeks) to emphasize glycinergic neurones and slightly counterstained with toluidine blue. *f*, Combined immunocytochemistry-autoradiography of neurotensin and ^3H -glycine uptake. Neurotensin-like immunoreactivity in the two major cell types encountered in turtle retina is indicated (A, B). Autoradiographic label is present over the type B cell showing that it also has a high affinity uptake system for glycine. The type A cell is not labelled. The section was exposed for 1 week and is not counterstained. *a-e*, $\times 318$; *f*, $\times 650$. Thickness of sections: *a-d*, 40 μm ; *e*, *f*, 0.5 μm .

Methods: For combined autoradiography and immunocytochemistry, turtles (20 cm shell diameter) were injected with 25 μl ^3H -glycine (NEN), resulting in an estimated concentration of 3 μM at the retinal level. After 4 h, the retinæ were fixed and processed for immunocytochemistry according to published procedures¹⁸. 40 μm -thick sections of resin-embedded retinæ were used to localize neurotensin-like immunoreactive neurones and these sections were then re-cut at 0.5–2 μm ¹⁹ for autoradiography. Intracellular microelectrodes filled with Lucifer yellow or horseradish peroxidase were used to record intracellularly from amacrine cells in an eye-cup preparation. The histological procedures after dye injection were similar to those described elsewhere^{9,20}.

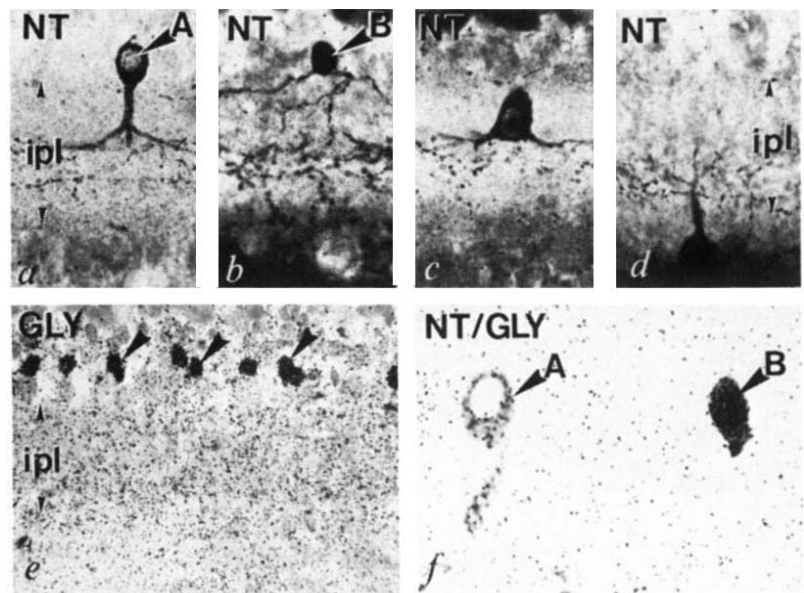
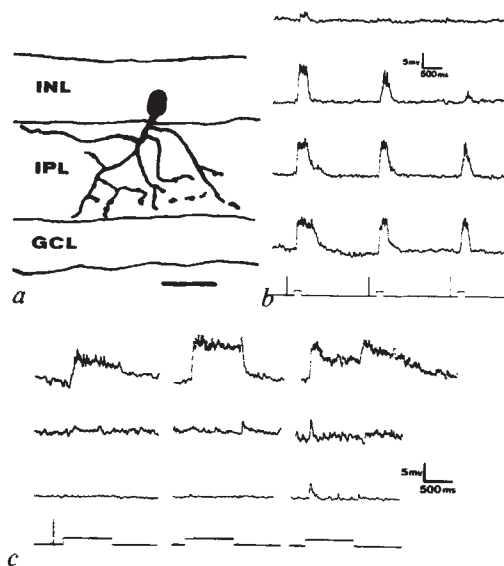


Fig. 2 *a*, Camera lucida drawing from transverse sections of an amacrine cell stained intracellularly with Lucifer yellow. Processes leave the soma close to the inner nuclear-plexiform layer border and ramify mainly in the proximal half of the inner plexiform layer. Scale bar, 25 μm . *b*, Photoresponses of the same cell to white light stimulation from a quartz-iodine lamp of increasing intensity (from left to right: 1.6×10^{-2} , 1.6×10^2 ergs $\text{s}^{-1} \text{cm}^{-2}$) and different size (from top to bottom: small spot 500 μm diameter, large spot 3,500 μm , annulus 3,500/1,200 μm). The signals were amplified with a WPI 707 amplifier and stored on a Rascal tape recorder. *c*, Photoresponses of the same cell to different monochromatic stimuli (small spot, from left to right: 625, 530, 450 nm). The irradiance was attenuated from top to bottom by 6, 4, 3 and 2 log units. The photoresponses are univariant with maximal sensitivity at 625 nm.



the inner plexiform layer, was identical to that of type B cells. They responded to light at the centre of their receptive fields with graded depolarizations (Fig. 2*b*). This depolarization was most prominent with a spot diameter of 500 μm and with the light intensity attenuated by 2.5 log units. Repetitive oscillations varying over 1–5 mV shaped the response. The centre response, including the oscillations, could be abolished almost completely by increasing the diameter of the illuminated area by up to 1,000 μm to stimulate both the centre and periphery of the cell's receptive field (middle trace, Fig. 2*b*). The persistence of only small 'on' or 'off' responses, depending on the light intensity, suggested the presence of an antagonistic surround, but peripheral illumination by itself generally produced no response. Monochromatic illumination evoked univariant responses (Fig. 2*c*), with maximal sensitivity around 625 nm. These physiological properties are similar to those attributed to diffuse amacrine cells described elsewhere⁹.

Our co-localization of neurotensin-like immunoreactivity and ^3H -glycine uptake may indicate that these two putative neurotransmitters, a neurotensin-like peptide and glycine, are released from the synaptic endings of type B cells in the inner plexiform layer. As such cells appear to be sustained-depolarizing amacrine cells with diffuse arborizations in the inner plexiform layer, these transmitters should be released in both

distal and proximal levels (sublaminae a and b) of the layer during depolarization induced by illumination of the receptive field centre.

We do not yet have evidence for a physiological action of both glycine and neurotensin in the turtle retina. In the mudpuppy retina, however, glycine inhibits both on and off ganglion cells, whereas neurotensin excites these cells^{12,13}. Because the dendrites of off-centre ganglion cells usually ramify in inner plexiform layer sublamina a and those of on-centre ganglion cells usually ramify in sublamina b^{14–16}, focal illumination should activate both neurotensin- and glycine-responsive mechanisms in both on- and off-centre ganglion cells. The electrophysiological findings in the mudpuppy retina are therefore in good agreement with the assumption of a release by a diffuse amacrine cell of neurotensin and glycine in both sublaminae.

If release and action of neurotensin and glycine within the turtle inner plexiform layer proves widespread, this would pose interesting questions about the role of neurotransmitters in visual information processing. Co-release as well as co-content of a peptide and a cooperatively acting conventional transmitter has been demonstrated elsewhere¹⁷, but co-release of antagonistic transmitters is unprecedented. The type B amacrine cells may be capable of multiple actions on postsynaptic neurones in the inner plexiform layer. This may enable a simple pathway to create a complex response pattern in postsynaptic neurones, thus considerably increasing the functional potentialities of the neural network of the inner plexiform layer.

This work was carried out in the laboratories of Dr William K. Stell. We thank Dr Stell for criticism of this work and for reading the manuscript. This work was supported by grants to W. K. Stell and A.K.B. from the MRC of Canada, W. K. Stell, A.K.B. and R.W. from the Alberta Heritage Foundation for Medical Research and to R.W. from the Swiss Academy of Medicine.

Received 23 March; accepted 13 September 1984.

1. Dowling, J. E. & Werblin, F. J. *J. Neurophysiol.* **32**, 315-338 (1969).
2. Dowling, J. E. & Ehinger, B. *Science* **188**, 270-273 (1975).
3. Brecha, N. in *Chemical Neuroanatomy* (ed. Emson, P. C.) 85-129 (Raven, New York, 1983).
4. Karten, H. J. & Brecha, N. *Nature* **283**, 87-88 (1980).
5. Stell, W. K., Marshak, D., Yamada, T., Brecha, N. & Karten, H. J. *Trends Neurosci.* **3**, 292-295 (1980).
6. Gilbert, R. F. T. & Emson, P. C. *Handbk Psychopharmac.* **16**, 519-556 (1983).
7. Hökfelt, T. et al. in *Neural Peptides and Neuronal Communication* (eds Costa, E. & Trabucchi, M.) 1-23 (Raven, New York, 1980).
8. Sternberger, L. A. *Immunocytochemistry* (Wiley, New York, 1974).
9. Marchiafava, P. L. & Weiler, R. *Proc. R. Soc. B* **214**, 403-415 (1982).
10. Kolb, H. *Phil. Trans. R. Soc. B* **298**, 355-393 (1982).
11. Eldred, W. D. & Karten, H. J. *J. comp. Neurol.* **221**, 371-381 (1983).
12. Dick, E. & Miller, R. F. *Neurosci. Lett.* **26**, 131-135 (1981).
13. Miller, R. F., Frumkes, T. E., Slaughter, M. & Dacheux, R. F. *J. Neurophysiol.* **45**, 743-763 (1981).
14. Weiler, R. & Marchiafava, P. L. *Vision Res.* **21**, 1635-1638 (1981).
15. Famiglietti, E. V., Kaneko, A. & Tachibana, M. *Science* **198**, 1267-1269 (1977).
16. Nelson, R., Famiglietti, E. V. & Kolb, H. *J. Neurophysiol.* **41**, 472-483 (1978).
17. Olschowka, J. A. & Jacobowitz, D. M. *Peptides* **4**, 231-238 (1983).
18. Eldred, W. D., Zucker, C., Karten, H. J. & Yazulla, S. *J. Histochem. Cytochem.* **31**, 285-292 (1983).
19. Stell, W. K. & Lightfoot, D. O. *J. comp. Neurol.* **159**, 473-502 (1975).
20. Weiler, R. *Cell Tissue Res.* **195**, 515-526 (1978).

Interactions between enkephalin and GABA in avian retina

Carl B. Watt, Ying-yet T. Su & Dominic Man-Kit Lam

Cullen Eye Institute, Baylor College of Medicine,
Houston, Texas 77030, USA

In addition to conventional neurotransmitters such as acetylcholine, dopamine, glycine and γ -aminobutyric acid (GABA)^{1,2}, a number of peptide-immunoreactive substances have recently been localized in the vertebrate retina^{3,4}. The functional roles of these retinal peptides and their interactions with conventional neurotransmitters are largely unknown. We have previously shown that exogenous opiates affect both the release of GABA and the firing patterns of ganglion cells in the goldfish retina⁵, and we have now begun a systematic characterization of the opioid pathways in the chicken retina, because, among vertebrate retinas, avian retinas contain the highest concentration of enkephalins⁶. Monoclonal antibodies specific for enkephalin have been used to demonstrate that a subpopulation of enkephalin-containing amacrine cells exists in the chicken retina^{6,7}. This retina also synthesizes Met⁵-enkephalin and releases it on cell depolarization^{6,8,9}. The enkephalin-induced inhibition of GABA release in goldfish retina⁵ led us to examine whether similar interactions occur in chicken, and if so, whether enkephalins and GABA coexist in the same amacrine cells. Our results, presented here, indicate that exogenous enkephalins do indeed inhibit GABA release in the chicken retina. Surprisingly, we found that although some amacrine cells contain both enkephalin and GABA, others contain only one or the other.

The effect of opiates on K⁺-stimulated release of pre-loaded GABA from GABA-accumulating amacrine cells of the chicken retina was examined by a method reported earlier^{5,10,11}. As exogenous GABA is taken up by both horizontal cells and subpopulations of amacrine cells in the retina¹², the amount of ³H-GABA accumulated into GABAergic horizontal cells was minimized by injecting ³H-GABA directly into the vitreous humour of the eye *in situ*^{11,12}. After 1 h, over 90% of the accumulated ³H-GABA was localized in GABA-accumulating amacrine cells (C.B.W., Y.-Y.T.S. and D.M.-K.L., unpublished result; see also ref. 11). The release of ³H-GABA from these cells by elevated external K⁺ (50 mM) in the absence or presence of opioid analogues could then be measured in isolated retina⁵. Met⁵-enkephalin inhibits K⁺-stimulated release of ³H-GABA in a dose-responsive manner (Fig. 1). This inhibition is mimicked by the enkephalin analogue [D-Ala²,D-Leu⁵]enkephalin (DADLE) and the opioid agonist morphine, but is reversed by the opioid antagonist naloxone. The maximal inhibition of ³H-GABA release by an opiate we tested was ~40%.

A logical extension of this study was to examine the anatomical relationships between putative enkephalinergic and GABAergic amacrine cells in this retina. A direct approach was to visualize enkephalinergic and GABA-accumulating neurones in the same retinal section. We used immunocytochemistry of enkephalin immunoreactivity to visualize the enkephalinergic neurones^{5,13-16}. The putative GABAergic neurones were localized by ³H-GABA uptake *in vitro* followed by autoradiography, an appropriate choice as in other retinas we have shown that neurones possessing a high-affinity mechanism for GABA uptake also contain the enzyme for GABA biosynthesis, and are therefore likely to be GABAergic^{17,18}. Thus, it is reasonable

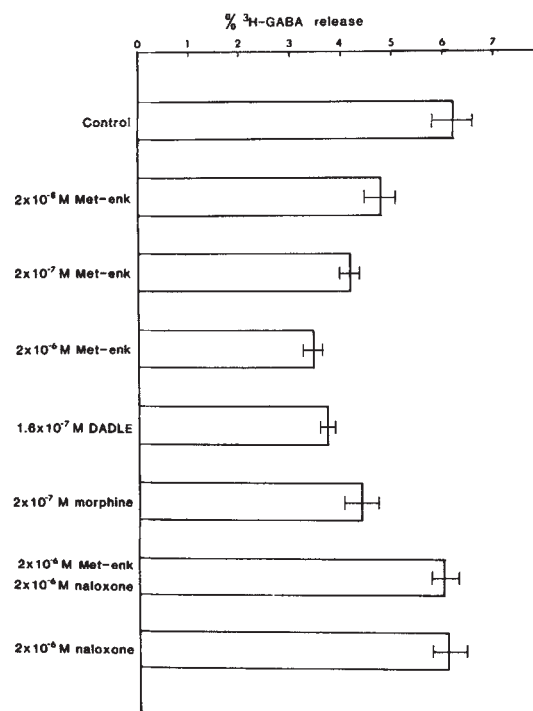
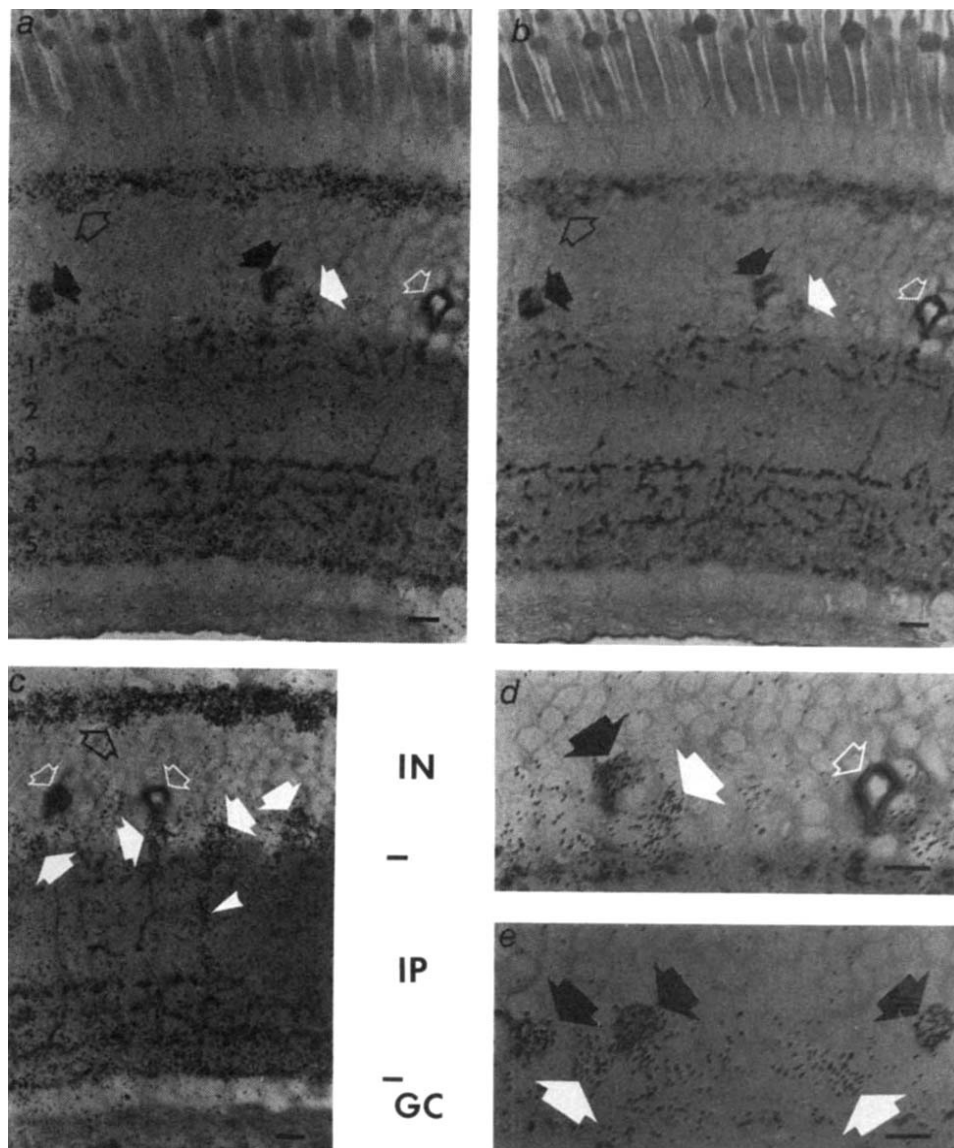


Fig. 1 Effects of opioid agonist and antagonist on K⁺-stimulated ³H-GABA release from GABA-accumulating amacrine cells of the chicken retina. One hour after an intravitreal injection *in vivo* with 50 μ l Ringer's solution containing 40 μ Ci ³H-GABA (specific activity 82.6 Ci mmol⁻¹), the injected eye was removed and the posterior eye cup bisected. The two pieces of retina were washed with excess oxygenated Ringer's solution for 30 min then incubated for 3 min with 1.5 ml of normal or K⁺-rich (50 mM) Ringer's solution in the absence or presence of an opioid analogue, using a method previously described. The figure shows the percentage of ³H-GABA released (radioactivity released/radioactivity present in the retina) in different conditions. Each bar represents the mean $\pm$ s.e.m. from at least three independent experiments of duplicate samples.

Fig. 2 Simultaneous localization of enkephalins (by immunocytochemistry) and ^3H -GABA uptake (by autoradiography) in the chicken retina. *a, b*, GABA-accumulating horizontal cells in chicken retina do not contain enkephalins. Double-labelled cells are denoted by solid black arrows; solid white arrows and open white arrows indicate cells labelled only for GABA or enkephalin, respectively. Open black arrows denote GABA-labelled horizontal cells. In *c* the white arrowhead indicates a GABA-labelled process within the inner plexiform (IP) layer. *d*, A higher magnification of the labelled cells in *a*. *e*, A higher magnification demonstrating typical double-labelled cells. IN, inner nuclear layer; GC, ganglion cell layer; 1–5 denotes distal to proximal sublayers of IP. Scale bar, 10 μm .

Methods: To visualize enkephalin-immunoreactive cells and GABA-accumulating neurones in the same retinal section, the retina was processed sequentially for autoradiography and immunocytochemistry. A piece of retina was isolated under room light and incubated for 10 min in 100 μl of an oxygenated Ringer's solution containing 10 μCi ^3H -GABA (specific activity 82.6 Ci mmol^{-1}). After incubation, the retina was rinsed for 1 min in unlabelled saline and placed in a cold fixative (4% paraformaldehyde/0.1% glutaraldehyde in 125 mM phosphate buffer, pH 7.3) for 1 h and transferred to a fresh fixative (4% paraformaldehyde) overnight at 4°C. Transverse sections (75 μm thick) of the ^3H -GABA-labelled retina were then cut with a vibratome and processed for enkephalin-immunoperoxidase staining at the light microscopic level. All steps were performed at room temperature and antibodies were diluted in phosphate-buffered saline (PBS, 20 mM phosphate buffer, 0.9% NaCl, pH 7.3) with 0.3% Triton X-100. Sections were first washed in 4% goat serum for 1 h, then incubated overnight in the primary antiserum (rabbit anti-Leu⁵-enkephalin; batch A-206 from K.-J. Chang^{6,15}) at a dilution of 1:1,000. After the incubation, the retinal sections were washed three times for 10 min with PBS and incubated in goat anti-rabbit IgG (1:20) for 3 h. After washing twice for 15 min in PBS, the sections were incubated in rabbit peroxidase-antiperoxidase (1:50) for 3 h, rinsed twice for 15 min in PBS, then transferred to the staining solution (10 ml PBS, 100 μl H_2O_2 , 30 μg diaminobenzidine 4-HCl) for 4–7 min. After the post-staining wash and light microscopic examination, the sections were fixed overnight with 2% glutaraldehyde in 125 mM phosphate buffer. Retinal sections were subsequently embedded in plastic¹³, and autoradiograms prepared routinely according to procedures described previously¹⁸.



to assume that neurones in the chicken retina which accumulated ^3H -GABA are probably GABAergic.

Examination of retinal sections processed sequentially for immunocytochemistry and autoradiography shows that the GABA-accumulating horizontal cells (Fig. 2*a, b*, open black arrows) in chicken retina do not contain enkephalins. Our double-label technique also reveals that some amacrine cells contain both enkephalins and GABA (Fig. 2, solid black arrows), whereas some GABA-accumulating amacrine cells (solid white arrows) do not contain enkephalins and some enkephalinergic neurones (open white arrows) apparently do not take up GABA.

The present study, together with our earlier finding⁵, indicates that in both chicken and goldfish retinæ, enkephalins inhibit the K^+ -stimulated release of GABA from putative GABAergic amacrine cells. Furthermore, electrophysiological studies of the goldfish retina⁵ suggest that opiate systems affect the information received by on-centre ganglion cells, by direct or indirect interactions involving enkephalinergic and GABAergic amacrine cells in the proximal region (sublamina b) of the inner plexiform

layer. In most vertebrate retinæ, sublamina b has been associated mainly with the processing of on-centre information, whereas the distal region (sublamina a) is involved with off-centre information^{19–21}. If this aspect of retinal organization is conserved in the chicken retina, then our observation that enkephalinergic and GABAergic processes overlap in the proximal region of the inner plexiform layer (Fig. 2) suggests that as in the goldfish retina⁵, interactions in the chicken retina may also influence the outflow of information to on-centre ganglion cells.

The finding that some GABA-accumulating amacrine cells in the chicken retina do not contain enkephalins is not surprising, as earlier studies have shown that although there was primarily one type of morphologically distinct enkephalin-immunoreactive amacrine cell^{6,7,13–16}, on the basis of somatic positions, there were several types of GABA-accumulating amacrine cells in the chicken retina^{6,12}. Similarly, the co-localization of GABA and enkephalins in the same amacrine cells is consistent with recent reports that certain peptides and transmitters coexist in the same neurones^{22–27}. In particular, enkephalin has been found in puta-

tive dopaminergic, serotonergic and cholinergic neurones²³, and enkephalin-immunoreactive substances have been recently detected in putative GABAergic neurones from cultured embryonic chick cerebral hemisphere²⁴.

The functional significance for peptide-transmitter coexistence is unknown, although numerous possibilities have been suggested²³⁻²⁸. One such suggestion is that in the parasympathetic system innervating the urinary bladder, enkephalin, which is co-localized with acetylcholine²⁵, may function as an inhibitory transmitter by blocking the presynaptic release of acetylcholine²⁶. Our finding that enkephalin inhibits GABA release from putative GABAergic amacrine cells is consistent with this hypothesis. However, our morphological findings suggest that enkephalin may also inhibit GABA release from putative GABAergic amacrine cells that do not contain enkephalin. Electron microscope studies involving the simultaneous visualization of ³H-GABA uptake and enkephalin immunocytochemistry are consistent with both mechanisms. Our preliminary results indicate that while there are synaptic structures which label for both enkephalin immunoreactivity and ³H-

GABA uptake, there also exist enkephalinergic processes which form synaptic contacts on ³H-GABA-accumulating profiles (C.B.W., unpublished observations). Thus, our results suggest that (1) at synapses where enkephalin and GABA are released from the same structure in response to depolarization, enkephalin might inhibit further GABA release at that synapse via a presynaptic mechanism and (2) at certain other synapses, enkephalin might diminish GABA release by interacting with opiate receptors located on neurones which contain GABA but not enkephalin. Probably, the most interesting conclusion from our data is that even within a single class of neurones such as the retinal amacrine cells, a neuroactive peptide and a neurotransmitter may coexist in some cells and be localized individually in different subpopulations.

We thank Ms Patricia Glazebrook and Mr Alex Kogan for technical assistance, Dr K.-J. Chang for anti-enkephalin antiserum and Ms Pat Cloud for typing the manuscript. This work was supported by grants from the US NIH (EY02490 to C.B.W., EY03701 to Y.-Y.T.S. and EY02432 to D.M.-K.L.) and the Retina Research Foundation (Houston).

Received 13 April; accepted 23 July 1984.

1. Lam, D. M. K. *et al. Neurochem. Int.* **1**, 183-190 (1980).
2. Ehinger, R. *Vision Res.* **23**, 1281-1291 (1983).
3. Stell, W., Marshak, D., Yamada, T., Brecha, N. & Karten, H. *Trends Neurosci.* **3**, 292-295 (1980).
4. Brecha, N. in *Chemical Neuroanatomy* (ed. Emson, P. C.) 85-129 (Raven, New York, 1983).
5. Djamgoz, M. B. A., Stell, W. K., Chin, C. A. & Lam, D. M. K. *Nature* **292**, 620-623 (1981).
6. Watt, C. B., Su, Y.-Y. T. & Lam, D. M. K. *Prog. Retina Res.* (in the press).
7. Watt, C. B., Tavella, D., Su, Y.-Y. T., Peng, Y. W. & Lam, D. M. K. *Soc. Neurosci. Abstr.* **9**, 282 (1983).
8. Su, Y.-Y. T. & Lam, D. M. K. *Soc. Neurosci. Abstr.* **9**, 289 (1983).
9. Su, Y.-Y. T., Su, S. & Lam, D. M. K. *Invest. Ophthalmol. vis. Sci.* **25** (Suppl.), 292 (1984).
10. Chin, C. A. & Lam, D. M. K. *J. Physiol., Lond.* **308**, 185-195 (1980).
11. Ayoub, G. S. & Lam, D. M. K. *J. Physiol., Lond.* (in the press).
12. Marshall, J. & Voaden, M. J. *Invest. Ophthalmol. vis. Sci.* **13**, 602 (1974).

13. Brecha, N., Karten, H. J. & Laverack, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3010-3104 (1979).
14. Tornqvist, K., Loren, I., Hakanson, R. & Sundler, F. *Expl Eye Res.* **33**, 55-64 (1981).
15. Fukuda, M. *Cell. molec. Biol.* **28**, 275-283 (1982).
16. Ishimoto, I. *et al. Invest. Ophthalmol. vis. Sci.* **24**, 879-885 (1983).
17. Lam, D. M. K. *et al. Nature* **278**, 565-567 (1979).
18. Brandon, C., Lam, D. M. K. & Wu, J.-Y. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3557-3561 (1980).
19. Famiglietti, E. V., Kaneko, A. & Tachibana, M. *Science* **198**, 1267-1269 (1977).
20. Stell, W. K., Ishida, A. T. & Lightfoot, D. O. *Science* **198**, 1269-1271 (1977).
21. Nelson, R., Famiglietti, E. V. & Kolb, H. J. *Neurophysiol.* **41**, 472-483 (1978).
22. Hokfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J. M. & Schultzberg, M. *Nature* **284**, 515-521 (1980).
23. Lundberg, J. M. & Hokfelt, T. *Trends Neurosci.* **6**, 325-333 (1983).
24. Louis, J. C. *et al. J. Neurochem.* **41**, 930-938 (1983).
25. Kawatani, M. *et al. Neurosci. Lett.* **39**, 143-148 (1983).
26. Konishi, S., Teunoo, A. & Oteuka, M. *Nature* **294**, 80-82 (1981).
27. Weiler, R., Ball, A. K. & Stell, W. K. *Soc. Neurosci. Abstr.* **9**, 896 (1983).
28. Jan, Y. N. & Jan, L. Y. *Trends Neurosci.* **6**, 320-325 (1983).

Expression of Ti plasmid genes in monocotyledonous plants infected with *Agrobacterium tumefaciens*

G. M. S. Hooykaas-Van Slogteren, P. J. J. Hooykaas* & R. A. Schilperoort

MOLBAS Research Group, Department of Plant Molecular Biology, Biochemical Laboratory, University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

When the bacterium *Agrobacterium tumefaciens* infects a fresh wound site on a dicotyledonous plant, it attaches to the plant cell wall and introduces a piece of its Ti plasmid DNA into the plant cell via an unknown mechanism¹⁻⁴. This piece of Ti plasmid DNA (the T-DNA) becomes integrated in the nuclear genome of the plant cell and is transcribed into a specific number of different transcripts. The T-DNA renders the plant cell tumorous, and also codes for enzymes involved in the synthesis of certain tumour-specific compounds called opines. Any DNA segment inserted into the T-region of the Ti plasmid by genetic manipulation seems to be co-transferred to the plant cell by *A. tumefaciens*. Thus, the Ti plasmid offers great potential as a vector for the genetic engineering of plant cells. However, as monocots are not susceptible to tumour formation by *A. tumefaciens*, it is generally believed that the Ti plasmid can be used as a vector for dicotyledonous plants only¹⁻⁵. This would severely limit the applicability of the Ti plasmid as a vector, as most commercially important crops are monocots. However, we report here data which indicate that the Ti plasmid may be a useful vector for transforming monocotyledonous plant species.

Infection of dicotyledonous plants with *A. tumefaciens* usually results in crown gall tumour formation, due to the expression of *onc* genes present in the T-DNA^{6,7}. These *onc* genes enable crown gall cells to grow in the absence of the phytohormones

auxin and cytokinin⁶⁻⁸. Recent evidence suggests that the *onc* genes in the T-DNA possibly code for enzymes that are directly involved in the biosynthesis of these hormones⁹.

Tumorous plants can be derived from certain tobacco crown gall lines which spontaneously regenerate shoots. These shoots do not usually form roots, but they can develop into mature plants after grafting onto normal tobacco understocks¹⁰. Superinfection of such plants with *A. tumefaciens* does not lead to tumour induction whereas infection with *A. rhizogenes*, which contains an Ri plasmid, results in the formation of an extensive swelling of the internodes having an infected wound rather than in the formation of a local tumour¹⁰. Similarly, infection of monocotyledonous plants with *A. tumefaciens* does not result in crown gall formation¹¹. It is believed there is no T-DNA transfer to the tumour plant cells^{12,13} or monocot cells¹⁴, either because the bacterium cannot attach to the plant cell walls^{12,14} or because of an abnormal phytohormone balance in such cells¹⁴. An alternative explanation is that T-DNA transfer did occur in these cases, but did not lead to tumour formation.

The opines octopine and nopaline are synthesized via enzymes that are encoded by T-DNA genes^{15,16} which only have eukaryotic regulatory sequences for expression¹⁷. The detection of opines in plant cells is therefore direct evidence for the presence of T-DNA. To establish whether T-DNA is still transferred to and expressed in tumorous plants and monocots after infection with *A. tumefaciens*, we assayed for octopine and nopaline in plant material from infected wound sites via the method described by Otten and Schilperoort¹⁸.

Grafted octopine tumour plants (from line TSO136)¹⁹ were infected with *A. tumefaciens* strain LBA2318 (containing a nopaline Ti plasmid), strain LBA1020 (an Ri plasmid), and strain LBA2347 (carrying a nopaline Ti plasmid and an Ri plasmid). Infection with LBA2318 did not result in any swelling or tumour formation, but after infection with LBA1020 or LBA2347 small swellings were apparent (Fig. 1). Functions determined by the Ri plasmid thus stimulate the occurrence of swellings. Nopaline was detected in plant material isolated from the swellings

* To whom correspondence should be addressed.

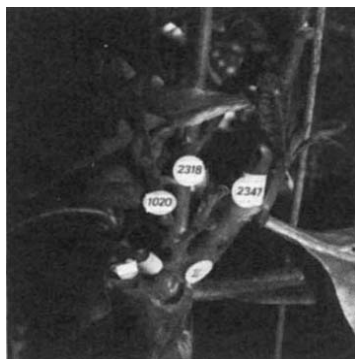


Fig. 1 Infection of a grafted crown gall shoot from line TSO136 with *A. tumefaciens* strains LBA1020, LBA2347 and LBA2318.

induced by LBA2347, which shows that the nopaline T-DNA from LBA2347 must have been introduced into the TSO136 crown gall cells. Therefore, we conclude that neither existing T-DNA, nor an altered phytohormone balance nor an altered cell wall are barriers to the transfer of T-DNA into the cells of the tumorous plant. The finding that introduction of a second copy of T-DNA into the tumorous shoots does not lead to tumour formation may be related to the abnormal phytohormone balance in the tumorous plants.

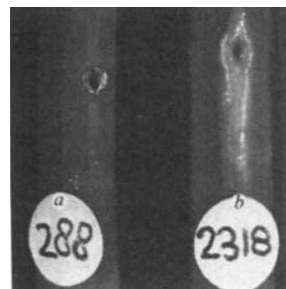
To determine whether monocots, which also do not show tumour formation after exposure to agrobacteria, can take up and express T-DNA after infection with *A. tumefaciens* we tested for production of opines in infected tissues. Two monocot species were selected for these studies, *Chlorophytum capense* (Liliaceae) and *Narcissus* cv. 'Paperwhite' (Amaryllidaceae). Plants of both species were infected with strain LBA2347. Fourteen days after infection small swellings were observed at the wound sites. After 21 days plant material from the wound sites always contained nopaline. In non-infected plant tissue neither octopine nor nopaline was detected. Following these positive results, infection tests were done on *Narcissus* cv. 'Paperwhite' with many different *A. tumefaciens* strains, LBA288 (Ti plasmid-cured; avirulent), LBA1516 (octopine Ti plasmid with a *virB* mutation; avirulent), LBA1010 (octopine Ti plasmid), LBA2318 (nopaline Ti plasmid), LBA1023 (Ri plasmid plus octopine Ti plasmid), LBA2347 (Ri plasmid + nopaline Ti plasmid). After infection with the avirulent strains LBA288 and LBA1516 no swellings were formed, and in plant cells from wound sites infected with these strains, neither octopine nor nopaline was detected. After infection with any of the other strains small swellings were invariably formed (Fig. 2). In the case of the infection with LBA1010 or LBA1023, octopine was detected in the plant cells from the wound sites; in the case of infection with LBA2318 or LBA2347, nopaline was detected.

These results show for the first time both the occurrence of T-DNA transfer to cells of monocotyledonous plants, and the expression of the T-DNA genes for octopine and nopaline synthase in such cells. This latter finding suggests that the regulatory sequences which are essential for transcription and translation of the octopine and nopaline synthase genes in cells of dicots also function in monocots. This result is of great importance in the development of vectors with selectable markers for monocotyledonous plants.

Our results show that T-DNA transfer to monocots occurs from strains with a wild-type Ti plasmid, but not from strain LBA1516, which has a defect in one of its essential *vir* genes. Therefore it is likely that *A. tumefaciens* introduces T-DNA into cells of monocots and dicots via the same mechanism. Some years ago it was found that the attachment of *A. tumefaciens* to the plant cell wall is necessary for the bacterium to accomplish T-DNA transfer²⁰. Our results suggest that *Agrobacterium* is able to attach to the cell walls of monocots. In support of this it has been shown recently that *A. tumefaciens* can attach to cells of *Asparagus officinalis* (a monocot)²¹ which had not previously been found for other monocots¹⁴. The apparent dis-

crepancy between these results might be explained by assuming that wounding can trigger transient changes in the cell walls of monocots which eventually enable *A. tumefaciens* to attach for a limited period of time.

Fig. 2 Infection of *Narcissus* cv. 'Paperwhite' with avirulent *A. tumefaciens* strain LBA288 (a) and with virulent strain LBA2318 (b). Opine assays were performed as described elsewhere¹⁸. A small piece of tissue (~60 mm³) was extracted with 30 µl cold buffer, and the supernatant was mixed 1:1 with buffer and incubated for 60 min. At the start ($t=0$) and end ($t=60$) of the incubation period, 2-µl samples from the mix were spotted onto electrophoresis paper. After electrophoresis the paper was dried then stained with phenanthrene quinone reagent. Yellowish spots of guanidine compounds (such as arginine, octopine and nopaline) were visualized under long-wave UV. Tissues from both monocots and dicots (used as a control) infected with the nopaline strains LBA2318 or LBA2347 normally displayed a spot corresponding to nopaline at $t=0$ and $t=60$. In monocot or dicot tissues from plants infected with the octopine strains LBA1010 or LBA1023, a clear spot corresponding to octopine was visible only in the $t=60$ sample. This shows that in both infected dicot and monocot cells sufficient nopaline, but not enough octopine, accumulates for direct detection at $t=0$. Although octopine is not detected at $t=0$, octopine synthase activity is present which converts *in vitro* pyruvate and arginine into octopine so that at $t=60$ an octopine spot becomes visible. Neither octopine nor nopaline synthase activity was present in uninfected tissues from monocots or dicots, nor in tissues from plants infected with the avirulent strains LBA288 or LBA1516.



crepancy between these results might be explained by assuming that wounding can trigger transient changes in the cell walls of monocots which eventually enable *A. tumefaciens* to attach for a limited period of time.

T-DNA transfer to monocots is not accompanied by the induction of large crown gall tumours. The finding that the T-DNA genes for octopine and nopaline synthase are expressed in monocotyledonous plant cells suggests that the other T-DNA genes are also expressed in the monocot cells. Therefore the absence of proper tumour formation in monocots may be because monocot cells (like the octopine crown gall cells) do not respond to the products derived from the T-DNA encoded *onc* genes, perhaps because of peculiarities of their phytohormone metabolism.

We suggest that the Ti plasmid may emerge as a useful vector for monocotyledonous plants of the families Liliaceae and Amaryllidaceae. Further research is needed to establish whether crops from other monocot families (for example, Gramineae) can be transformed by the Ti plasmid. Nevertheless, our results have considerable implications for the biotechnology of a vast group of plant species of commercial interest.

We thank Drs A. Hoekema, L. Melchers and R. J. M. van Veen for critical reading of the manuscript.

Received 18 June; accepted 30 July 1984.

- Kahl, G. & Schell, J. (eds) *Molecular Biology of Plant Tumors* (Academic, London, 1982).
- Hooykaas, P. J. J. & Schilperoort, R. A. *Adv. Genet.* **22**, 209-283 (1984).
- Ream, L. W. & Gordon, M. P. *Science* **218**, 854-859 (1982).
- Schell, J. & Van Montagu, M. *Bio/Technology* **1**, 175-180 (1983).
- Flavell, R. & Mathias, R. *Nature* **307**, 108-109 (1984).
- Ooms, G., Hooykaas, P. J. J., Moolenaar, G. & Schilperoort, R. A. *Gene* **14**, 33-50 (1981).
- Garfinkel, D. J. *et al. Cell* **27**, 143-153 (1981).
- Braun, A. C. *Proc. natn. Acad. Sci. U.S.A.* **44**, 344-349 (1958).
- Schröder, G., Waffenschmidt, S., Weiler, E. W. & Schröder, J. *Eur. J. Biochem.* **138**, 387-391 (1984).
- Wullems, G. J., Molendijk, L., Ooms, G. & Schilperoort, R. A. *Cell* **24**, 719-727 (1981).
- De Cleene, M. & De Ley, J. *Bot. Rev.* **42**, 389-466 (1976).
- Lippincott, J. A. & Lippincott, B. B. *Science* **199**, 1075-1078 (1978).
- Krens, F. A., Wullems, G. J. & Schilperoort, R. A. in *Structure and Function of Plant Genomes* (eds Ciferri, O. & Dure, L.) 387-408 (Plenum, New York, 1983).
- Rao, S. S., Lippincott, B. B. & Lippincott, J. A. *Physiologia Pl.* **56**, 374-380 (1982).
- Schröder, J. *et al. FEBS Lett.* **129**, 166-168 (1981).
- Murai, N. & Kemp, J. D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 86-90 (1982).
- De Greve, H. *et al. J. molec. appl. Genet.* **1**, 499-512 (1983).
- Ottens, L. A. B. M. & Schilperoort, R. A. *Biochim. biophys. Acta* **527**, 497-500 (1978).
- Van Sloteren, G. M. S., Hoge, J. H. C., Hooykaas, P. J. J. & Schilperoort, R. A. *Plant molec. Biol.* **2**, 321-333 (1983).
- Lippincott, B. B. & Lippincott, J. A. *J. Bact.* **97**, 620-628 (1969).
- Draper, J., MacKenzie, I. A., Davey, M. R. & Freeman, J. P. *Plant. Sci. Lett.* **29**, 227-236 (1983).

Defending the Earth

Eric Ashby

International Environmental Policy: Emergence and Dimensions.

By Lynton K. Caldwell.

Duke University Press: 1984. Pp.367. Hbk \$37.50, £28.50; pbk \$14.75, £11.90.

ON 1 January 1970 the word "biosphere" was incorporated into the public laws of the United States of America. The word appears in the preamble to the Environmental Policy Act, 1969. It was one of many incidents — Britain's Wildlife and Countryside Act 1981 is another — in what some people call a "new environmental paradigm": a worldwide change in assumptions and beliefs about our responsibility for the rest of nature.

Are such incidents evidence of the emergence of a new paradigm? Lynton Caldwell believes that they are, and that is what this book is about. No one is better qualified than he to write such a book because he combines a capacity to digest huge masses of published material (the references in this book cover 56 pages of small print) with a capacity to write clearly and pungently. He has already described the gestation and birth of a national decision, the law to protect the environment of the United States (*Science and the National Environmental Policy Act*, reviewed in *Nature* 303, 354; 1983). Now he takes a far wider view and offers a "sketch-description" of international decisions about environmental policy.

Caldwell himself would wish the book to be called only a sketch-description. He emphasizes that he writes at a high level of generalization and that a detailed analysis would require many volumes. It would be dull, too, and Caldwell's account is not. The book can be put in perspective by recalling something Caldwell wrote over a decade ago. Americans (and it is true for Europeans too) live in a "hypertrophic society":

The hypertrophic society has fallen victim to a social 'disease', an 'endocrine failure' followed by runaway metabolism and accelerating growth. The negative feedback and homeostatic mechanisms that contain and project a stable self-renewing society have been supplanted by the self-generating impetus of positive feedback [*National Resources Journal*; Vol. 11, No. 3 (1971)].

Social carcinoma: a grim diagnosis. Caldwell's present theme is that a minority of thoughtful people all over the world are now aware of this "disease" and their influence is sufficient to persuade governments to take steps, even at an international level, to arrest its erosion of the biosphere. His book describes these international conferences, treaties, agreements, declarations; and he evaluates them with detachment.

The environmental movement, which

(Caldwell thinks) recruits more people than join the world's peace movements, is an example that not only individuals but whole nations can learn from experience. People learn about something from press and television (the link between sulphur emissions and acid rain, for instance). It may then become an "issue". This in turn brings pressures upon governments to do something about the issue, at first at national level to satisfy self-interest and

P.L. 91-190 LAWS OF 91ST CONG.—1ST SESS. Jan. 1

NATIONAL ENVIRONMENTAL POLICY ACT OF 1969

For Legislative History of Act, see p. 2751

PUBLIC LAW 91-190; 88 STAT. 852

(S. 1075)

An Act to establish a national policy for the environment, to provide for the establishment of a Council on Environmental Quality, and for other purposes.
Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That:
This Act may be cited as the "National Environmental Policy Act of 1969".

PURPOSE

Sec. 2. The purposes of this Act are: To declare a national policy which will encourage productive and enjoyable harmony between man and his environment; to promote efforts which will prevent or eliminate damage to the environment and biosphere and stimulate the health and welfare of man; to enrich the understanding of the ecological systems and natural resources important to the Nation; and to establish a Council on Environmental Quality.

Law of change? The opening passages of the National Environmental Policy Act, which came into effect 15 years ago.

then, though much less effectively, internationally. That there has been any success at all at international level is remarkable when one considers how intense is the dislike, suspicion and jealousy even between nations that are political allies. The only justification for hoping that international co-operation may become less difficult lies in the evidence summarized in Caldwell's book, fortified by a faith that the environmental movement is not a transient enthusiasm but "an awakening of modern man to a new awareness of the human predicament on earth".

We have heard all this before, in the overwrought style of *Blueprint for Survival*. But the contrast between that sort of clamorous rhetoric and Caldwell's calm diagnosis is as wide as the contrast between a layman's guess and a medical consultant's considered opinion. Let me first say something about the facts in Caldwell's book and then offer an opinion about the faith needed to interpret the facts.

The prime fact was the United Nations Conference on the Human Environment, in Stockholm in 1972. The importance of

this was not the meeting itself but the decades of activity preceding it; that is what made a conference inevitable. The conference itself was tedious and at times degenerated into political wrangling. But it had two important consequences: the first, to put environmental policies permanently on the agenda for national and international discussion; the second to create the United Nations Environment Programme (UNEP) which, although its achievements over the past ten years have been modest, has undoubtedly helped to bring environmental policies within the ambit of international law.

Since 1972 millions of man-hours have been spent and tons of print have been produced to promote international environmental policy. You can get an idea of the scale of effort from the list of bodies (all with acronyms conveniently listed in the book) concerned with the issues: there are over 200 of them. Some promising agreements have been reached at a regional level: the environmental directives that are complied with (more or less) by member states in the European Community are an example. And on paper, at any rate, there are numerous treaties, for instance to control endangered species, to conserve wetlands used by migrating birds, to stiffen protection against products harmful to health (a United Nations Resolution (37/137) adopted in 1981 with one dissident: the United States!), to monitor long-range air pollution, to prevent pollution from ships, to conserve the Antarctic.

But does all this paperwork really mean that the paradigm is changing? Yes-and-no is the answer I gather from Caldwell's book. Yes, because this is just the kind of activity one expects before a paradigm-shift and it has no precedent. No, because international law and policing cannot alone cope with the issues; there will need to be a massive consensus which puts long-range interests before short-term convenience, and as yet there is no sign of that.

This is how Caldwell points to the problem.

For as long as it pays people to destroy living systems unnecessarily, to deplete nonrenewable resources, and to degrade the quality of the biosphere, they may be expected to do so except as influenced by social or political constraints.

He has no patience with reports that are "long on 'musts' and 'shoulds' and very short on 'hows'". But he would surely agree that he is pretty short on "hows" himself, though he does encourage those who have seen the light to press on, regardless of setbacks, to learn by experience and to recruit supporters. A radical, lasting shift in attitudes may take a century; progress in any one lifetime may seem disheartening; but we have had an International Geophysical Year, a Global Atmospheric Research Programme, and in many nations already there is a public conscience about the environment which

politicians and industrial corporations disregard at their peril.

It is to these signs of glacial change that Caldwell attaches his faith. But it is a sombre faith, laced with doubt. Here (on p. 274) is his conclusion:

Barring unforeseen events, the ecological quality of the environment for all living things seems almost certain to suffer a net decline in the decades ahead....It is difficult to believe that a change in human perceptions and values could occur on a scale and within a period of time to reverse this pessimistic conclusion. Yet human history has recorded abrupt and unpredicted changes in social behavior. Events of the decade 1972-82 suggest that peoples and their governments are...developing an appreciation of the consequences of continuing along the path of ecologically heedless exploitation of the earth.

And if they do heedlessly continue? In the essay I quoted at the beginning of this review Caldwell gives his answer. The task, he wrote, is to cure the patient of the disease of societal hypertrophy. If:

we are unable to avoid disaster, some moral satisfaction may be gained by the knowledge that our failure was not a failure of nerve or will...the outcome may be tragic; but it will be neither disgraceful nor absurd.

Here is encouragement to continue what Caldwell calls the "Defense of Earth".

Lord Ashby is a Fellow of Clare College, Cambridge. He was Chairman of the Royal Commission on Environmental Pollution from 1970 to 1973.

ADVERTISEMENT

Aquaculture and Fisheries Management

Edited by D. H. Mills and R. Roberts and published quarterly. Subscription rates for 1985 are £60.00 (UK), £72.00 (overseas), \$108.00 (USA and Canada) post free.
0266 - 996X

This new journal has been developed from Fisheries Managements and is an international journal which will publish research papers and short communications on all aspects of aquaculture and fisheries management including practical and theoretical papers on the conservation of fresh water, estuarines and marine fish, the creation, development or management of commercial fresh-water, estuarine and marine fisheries in all parts of the world.

Blackwell Scientific Publications Ltd.
P.O. Box 88
Oxford

Place for biography in chemistry

Colin A. Russell

Amedeo Avogadro: A Scientific Biography.

By Mario Morselli.

Reidel: 1984. Pp. 375. Dfl. 160, £40.75, \$59.50.

Boussingault: Chemist and Agriculturist.

By F.W.J. McCosh.

Reidel: 1984. Pp. 280. Dfl. 140, £35.50, \$53.50.

CHEMICAL biography is not much in fashion today. There was a time when history of chemistry dominated history of science, but for some years the pendulum has swung far in other directions, especially towards the ubiquitous social history of scientific ideas and institutions. As for biography, many potential writers have been frozen into immobility by fears of perpetuating the cult of the scientific "hero" which, in these iconoclastic and egalitarian days, wouldn't do at all.

Yet biography is not hagiography and there still is a place for studies of our chemical forefathers. Indeed it is now necessary to replace the ageing monographs of a previous generation and to deploy the resources and display the insights of modern scholarship. Hence Reidel's conception of a series of books on "Chemists and Chemistry" is timely and welcome. The first two volumes are about the Italian aristocrat whose hypothesis was once familiar to every elementary student of chemistry, Amedeo Avogadro (1776-1856), and the French chemist J. B. J. D. Boussingault (1802-1887). If the order is alphabetical we shall have to wait some time for the volume on Zsigmondy.

In the first two books of any new series it would be hard to find a pair that differ more strikingly than these. At an editorial level alone they are far from homogeneous; one has American spelling and notes at the ends of chapters; the other uses English spelling and consigns notes to the back of the book. The systems of referencing are also entirely different, as are the use of page-headings and various other details. Perhaps only the fastidious will care for such things, but no one can fail to notice considerable differences of treatment. In part, no doubt, this springs from the subjects themselves. Boussingault, relatively unknown to all but a handful of historians, is chiefly memorable for his contributions (that is the right word) to agricultural science. Avogadro has a certain deathless reputation for enunciating the hypothesis connecting gas volumes and molecular numbers, so pointing a way out of the impasse over atomic weights that plagued chemistry for over half a century.

In his long-awaited volume, Morselli

first traces the complicated political history of Piedmont where for centuries Avogadro's ancestors had lived. Though geographically part of Northern Italy it was, for most of Avogadro's life, part of the far-flung Kingdom of Sardinia, as well as being for some years a dependency of Napoleonic France. We read of his legal studies at Turin during which he was seduced into science, and of his appointment to chairs in physics and mathematics at, first, Vercelli, and then Turin itself. The scene being set we are next conducted, chapter by chapter, on a tour of Avogadro's scientific papers and their subsequent reception (or lack of it). As the subtitle indicates this is a *scientific* biography. After the first chapter little is heard of the political, social and cultural roots of the science described and there is no systematic attempt to relate them. This is consistent with the author's declared perception of dangers in "overly relating them [scientific theories] to earlier philosophical and cultural movements" (p. 356).

By contrast McCosh gives a more or less chronological account of Boussingault's life, from his childhood in war-torn Paris, through his unconventional scientific education at a mining school near Lyon to his adventures as teacher and scientific consultant in South America. He later became a collaborator of Dumas at the Sorbonne, professor at the Conservatoire des Arts et Métiers in Paris and pioneer in experimental farming in Alsace. He also worked in metallurgy, air and water analysis and on the thermal decomposition of barium peroxide. All this, and more, is described in detail and the social and scientific elements are woven into one tapestry. However, even this approach does not always show how Boussingault's science reflected social and other values and one is often left with more questions than answers. One welcome emphasis is on education, both given and received. The conclusion that Boussingault failed to become "a truly great chemist" because "he suffered from the lack of a university training" (p. 13) is, however, much too simple. It implies a systematic approach that was by no means always provided by universities, and neglects the many highly successful chemists who were denied any kind of undergraduate teaching.

Both Morselli and McCosh have written thorough, well-researched books. The adventures of Boussingault in South America are particularly well-told and should be of interest to many political historians who do not habitually read *Nature*. All who teach chemistry at school and have to conjure with the name of Avogadro will find Morselli's book an admirable corrective to popular errors. Such include the notion that Avogadro's solution to the atomic weight problem was immediately accepted by grateful colleagues, or (contrariwise) that he was totally ignored in his lifetime because he

was a lawyer, inarticulate or Italian. Nor can the howlers be perpetuated that he proposed in 1811 diatomic molecules for the common elementary gases, or that he was instantly rescued from oblivion by Cannizzaro at the Karlsruhe Congress of 1860.

Of these two books Morselli's makes the more telling points because the scientific and historiographic issues have such unusual complexity and importance. But it has less relevant and fewer illustrations than its companion volume and a number of minor textual irritations. Thus Davy (whom McCosh usually calls "Humphrey") was not a single-minded supporter of contact electrification (Morselli, p. 63), nor was he a lifelong disciple of Boscovich (p. 199). Kekule was certainly not a "close friend" of Frankland

(p. 233), was not called "Frederick" and did not produce the first edition of his *Lehrbuch* in 1867 (p. 266). Prout's *Bridge-water Treatise* appeared in 1834 (p. 182), not 1904 (p. 184).

It is to be hoped that future volumes in the series will benefit from more careful editorial attention. In one chapter of Morselli, for example, an article by Graebe is given no less than five different abbreviated titles, while McCosh's book is marred by an extremely high number of misprints. Other than these blemishes the main deterrent is the price. But to those who can afford them both books have much to tell of the rise of modern chemistry. □

Colin Russell is Professor of History of Science and Technology at the Open University.

Pieces of artificial intelligence

Wendy G. Lehnert

Artificial Intelligence: Tools, Techniques, and Applications.

Edited by Tim O'Shea and Marc Eisenstadt.

Harper & Row: 1984. Pp.497. Pbk \$19, £12.95.

TRUE to their title, O'Shea and Eisenstadt have produced an anthology of papers dealing with the tools, techniques and applications of artificial intelligence. However, while the book is intended to serve as an introduction to the field, it should not be seen as a textbook. The 15 chapters have been written by different authors and no effort has been made to achieve a unified effect, each contribution being a self-contained introduction to its own topic. As such, therefore, the book cannot be compared to single-author texts, for example Patrick Winston's *Artificial Intelligence* (published by Addison-Wesley in 1984), nor does it attempt to provide the comprehensive coverage of the three volumes of *The Handbook of Artificial Intelligence* by A. Barr, P. Cohen and E.A. Feigenbaum (Pitman, 1981 and 1982). But viewed as a collection of introductory papers, it is reasonably solid.

Here students of AI will find well-presented material that is certain to motivate further delving into the literature. Among the topics covered are natural language, vision, robotics and expert systems, as well as introductions to the LISP and PROLOG programming languages. There is, too, an account of text processing, which really has nothing to do with AI but does show how AI techniques can be useful in spin-off applications.

There is also a chapter on how to get a PhD in AI, apparently aimed at graduate students who have no adviser. As a teacher,

I found this contribution laudable in intent but irresponsible in practice. It gives the impression that a PhD in AI can be pursued by anyone with sufficient interest in the subject and access to adequate computing facilities. In fact, there are only a few computer science departments capable of supporting quality research in AI, and all such research is conducted in the context of organized projects. Since there is a very high demand for well-qualified people in AI, and a paucity of such people to go around, there is a grave danger that third-rate research will be accepted at lesser institutions for the sake of exploiting the bullish job market. A trend in this direction could hurt AI irreparably by destroying what delicate credibility has been established by more responsible research sites: a field that is as controversial and susceptible to media hype as AI cannot afford to make room for mediocre practitioners. So I fail to see how this chapter will help anyone, and I fear it will only serve to encourage borderline research.

That chapter apart, my reaction to the collection is largely positive. The various contributions grew out of a school which was aimed at both academic and industrial participants, so the book should be read in the same spirit with which one approaches a conference proceedings volume. The quality varies from paper to paper and readers are left to discover for themselves any unifying threads between topics. But given additional guidance to make more explicit the underlying principles of AI, the book does provide a good introductory background to the field. I would therefore suggest that teachers use it as a supplementary text, or as a source of selected readings, and it should certainly be included in any library intending to keep a comprehensive collection of the AI literature. □

Wendy G. Lehnert is an Associate Professor of Computer and Information Science at the University of Massachusetts.

Spasm? Or rigor?

Michael Spencer

Contractile Mechanisms in Muscle.

Edited by G.H. Pollack and H. Sugi.
Plenum: 1984. Pp. 921. \$125, £106.25.

IN THE heady years of the 1960s, D.R. Wilkie advertised a lecture on muscle with a jokey prospectus that started: "Available now. LINEAR MOTOR. Rugged and dependable: design optimized by worldwide field testing over an extended period" and ended with the laconic phrase "Good to eat". Optimism was in the air. Nobody knew exactly what made the filaments slide, but there was no shortage of ingenious hypotheses. As few of these could immediately be tested, imaginations roamed freely; among ideas discussed were crossbridges on springs, erectile crossbridges, contractile filaments and various wheezes involving forces at a distance.

As the 1970s dawned, the rocking crossbridge model emerged as front-runner with influential backing from the two Huxleys and others. It looked ideally suited to the simple four-stage kinetic scheme of Lymn and Taylor, with a powered change in the angle of attachment corresponding to one of the steps. It seemed inevitable that time-resolved studies by X-ray and other techniques would soon sort it all out, and Nobel-spotters made mental bets on the likely beneficiaries.

Then troubles began to mount, and nagging doubts to arise. X-ray work of ever-increasing sophistication and cost failed to reveal any obvious correlation with kinetics; and the kinetic schemes themselves became so complex that E.W. Taylor once started a lecture by observing drily that we now knew less than we did two years earlier. Worst of all, a series of new experiments with fluorescent and EPR probes failed to provide any consistent evidence that the crossbridges rocked at all. Heretics who had temporarily been silenced began to raise their voices: was the supposed mainstream of muscle research really a blind channel? At the present time few workers are sure of anything any more, and there is a desperate feeling that although no alternative scheme is highly plausible, there is some vital feature missing from the crossbridge hypothesis.

Young post-doctorals just entering the field are less afflicted by self-doubt than their more world-weary elders, and for them there is no shortage of work: many types of muscle remain almost virgin territory, and there are fascinating new techniques to spend money on. So the flow of bulky conference reports continues unabated, and the volume under review is typical: it records the second international symposium on crossbridge mechanisms and contains around half-a-million words, ending with a ruminative summary (expressing due gratitude to the organizers)

INSERM

Institut National de la Santé et de la Recherche Médicale

Cours pratique de génie génétique Constitution de banques d'ADN génomique à l'aide de phages ou de cosmides

Edited by S. Wain-Hobson,
C. Bishop et A. Dejean
1984, Vol. 118, 88 pages,
60 FF H.T.

Nouvelles techniques d'étude d'hémostase et de thrombose

Edited by S. Levy-Toledano
1984, Vol. 120, 128 pages,
75 FF H.T.

Méthodologie des études épidémiologiques et cliniques sur le diabète

Edited by Laure PAPOZ
1984, Vol. n° 122, 264 pages,
90 FF H.T.

Naître en France

Editeb by
C. RUMEAU-ROUQUETTE,
C. du MAZAUBRUN,
Y. RABARISON



1^{er} titre de la collection
«Grandes enquêtes»
Coédition INSERM/DOIN
240 pages, 102,80 FF H.T.

LES EDITIONS
INSERM

101, rue de Tolbiac
75654 Paris Cedex 13
France
Tél. : 584.14.41

Prepayment is required Bank cheque
to the order of INSERM

by one of the grand masters of the field. The fact that the conference took place more than two years ago is, however, significant: the reprehensible delay in publication has not in fact rendered most of the material obsolete, because progress just isn't that fast any more.

The main topics covered by the meeting were structural dynamics, including the curious affair of the shortening thick filaments in *Limulus*; mechanics, particularly tension transients and stiffness; and energetics. The text is enlivened by reports of verbal exchanges, such as this baffling dialogue:

A: Where you say A-band mass, you don't actually mean "mass" do you?

B: What would you have me say?

A: Whatever you mean — what do you mean? or this outburst from an exasperated critic:

X: I mean you've just done the control, got the wrong results, and then persist in fitting your hypothesis?

Y: No . . .

A lot of effort at the conference went into reconciling the evidence from spectroscopic probes with the rocking crossbridge model: if the probes showed no motion, could they all have been attached (by sheer bad luck) to a portion of the myosin head that remained fixed to actin, while another unlabelled portion did the rocking? The need for detailed crystallographic structures of the relevant proteins was heavily emphasized; and there is now (two years later) a glimmer of hope that this may be achieved before the retirement dates of those who have devoted

their lives to the crossbridge hypothesis.

It would be a mistake to belittle this obsession with molecular detail by comparing it with the fruitless debates of mediaeval theologians, as Graham Hoyle tends to do in his recent book (*Muscles and their Neural Control*, published by Wiley in 1983). If (dreadful thought) the crossbridge head turns out not to be the source of motion after all, then belated and more widespread attention will have to be given to iconoclastic ideas such as W.F. Harrington's contractile S-2 region, or to charge-effect hypotheses of the kind adhered to by G.F. Elliott. Neither of the last-named workers presented papers at the conference in question, but occasional references to their thoughts appear in the proceedings like Banquo's ghost at the feast in *Macbeth*.

It is imperative to resolve the uncertainties — not just for the satisfaction of those involved with muscle, but because motility is now seen to be of such universal importance in cell biology. The muscle proteins or their analogues are so widely distributed among cell types that a single basic mechanism seems rather likely, even though control systems vary considerably. It remains a central problem to find out exactly what goes on, and the present uncomfortable state of spasm in the field must surely soon be succeeded by purposeful movement. □

Michael Spencer has just given up being a Senior Research Fellow in the Department of Biophysics at King's College, University of London.

Starting with laser spectroscopy

G.R. Wilkinson

Foundations of Laser Spectroscopy.

By Stig Stenholm.

Wiley: 1984. Pp. 268. £40.95, \$36.95.

ALTHOUGH the laser was invented before most of our undergraduates and post-graduates were born, laser spectroscopy is still a young and active area of research. Hence Dr Stenholm's book, which expounds upon the foundations of the subject, is likely to be widely read.

The author starts by establishing a notation and recalls the basic principles of electrodynamics and quantum mechanics. Following this introductory material is a chapter in which the response of the laser medium to strong electromagnetic fields is derived, and another which treats the physical basis of laser operation (this latter account might have been more useful if consideration of the working of some actual lasers had been included). The next chapter contains description of some applications to laser spectroscopy; here I found the treatment rather abstract for the

average reader, who will have to look elsewhere for a review of the spectroscopy involved in lasers. Fluctuations are concisely introduced in Chapter 5, whilst finally field quantization and some of the simplest consequences are discussed. The book contains helpful comment and references at the end of each chapter.

The author, a theoretical physicist,

intends this volume for beginning theorists and experimentalists who lack extensive training in algebraic manipulations, hence it contains more equations than are needed for readers more accustomed to mathematical sophistication.

This is indeed one of the book's most valuable features; however I am not sure that the claim that it can be used as a textbook for a course, even without previous knowledge of laser physics, is valid. For example it is disappointing to find that the author has left out mention of processes belonging to non-linear optics as these are so prominent at the present time.

Despite the misgivings that I have as a spectroscopist, this is undoubtedly an important book, and one which will not date quickly. Every worker in the field of laser spectroscopy should have access to a copy. □

G. R. Wilkinson is Professor of Physics at King's College, University of London.

Technology

- Advances in Cryogenic Engineering**, Vol. 29. R. W. FAST (ed.). Plenum: 1984. Pp. 1049. ISBN 0-306-41703-0. Np.
- Advances in Energy Systems and Technology**, Vol. 4. PETER AUER and DAVID DOUGLAS (eds). Academic: 1983. Pp. 213. ISBN 0-12-014904-4. \$49.
- First European Conference on Coal Liquid Mixtures**. INSTITUTION OF CHEMICAL ENGINEERS. Pergamon: 1983. Pp. 415. ISBN 0-08-031397-3. £27, \$48.50.
- Heterogeneous Reactions: Analysis, Examples, and Reactor Design**, Vol. 2 Fluid-Fluid-Solid Reactions. By L.K. DORAISWAMY and M.M. SHARMA. Wiley: 1984. Pp. 374. ISBN 0-471-05367-8. £59.35.
- Ion Exchange Technology**. D. NADEN and M. STREAT (eds). Ellis Horwood: 1984. Pp. 742. ISBN 0-85312-770-0. £45.00.
- Rainfall as the Basis for Urban Run-off Design and Analysis**. Water Science & Technology, Vol. 16, Nos 8-9. PETER HARREMOES (ed.). Pergamon: 1984. Pp. 368. ISBN 0-08-031506-2. £50.00.
- River Basin Management**. Water Science & Technology, Vol. 16, Nos 5-7. D.H. NEWSOME and A.M.C. EDWARDS (eds). Pergamon: 1984. Pp. 670. ISBN 0-08-031505-4. £75.00.
- Robots and Telechairs: Manipulators with Memory; Remote Manipulators; Machine Limbs for the Handicapped**. By M.W. THRING. Ellis Horwood: 1983. Pp. 298. ISBN 0-85312-274-1. £27.50.
- THRING. Ellis Horwood: 1983. Pp. 298. ISBN 0-85312-274-1. £27.50.
- The Silicon Idol: The Micro Revolution and its Social Implications**. By MICHAEL SHALLIS. Oxford University Press: 1984. Pp. 188. ISBN 0-19-215877-5. £8.95.
- Stepping Motors and their Microprocessor Controls**. By TAKASHI KENJO. Oxford University Press: 1984. Pp. 244. ISBN 0-19-859326-0. £25.
- Treatise on Materials Science and Technology**, Vol. 19 Experimental Methods Part B. HERBERT HERMAN (ed.). Academic: 1983. Pp. 274. ISBN 0-12-341842-9. \$45.

Computer Science

- The Computer Comes of Age: The People, the Hardware, and the Software**. By R. MOREAU. MIT Press: 1984. Pp. 227. ISBN 0-262-13194-3. £17.95.
- Computing in Applied Science**. By WILLIAM J. THOMPSON. Wiley: 1984. Pp. 325. ISBN 0-471-09355-6. £26.70.
- The Electronic Library**. By KENNETH E. DOWLIN. Mansell Publishing: 1984. Pp. 199. Pbk ISBN 0-918212-75-8. Pbk £21.
- The Robot Revolution**. By TOM LOGSDON. Simon & Schuster: 1984. Pp. 207. Pbk ISBN 0-671-46705-0. Pbk \$9.95.
- SPSS/PRO For Dec Professional 350**. McGraw-Hill: 1984. Pp. 282. ISBN 0-07-046556-8. Np.
- Super-Computers and Parallel Computation**. D.J. PADDON (ed.). Oxford University Press: 1984. Pp. 258. ISBN 0-19-853601-1. £25.
- Text Processing**. Cambridge Computer Science Texts 20. By A. COLIN DAY. Cambridge University Press: 1984. Pp. 141. Hbk ISBN 0-521-24432-3; pbk ISBN 0-521-28683-2. £14, \$29.95; pbk £5.95, \$12.95.

Biological Sciences

- Advances in Lipid Research**, Vol. 20. RODOLFO PAOLETTI and DAVID KRITCHEVSKY (eds). Academic: 1983. Pp. 367. ISBN 0-12-024920-0. \$48.50.
- Advances in Metabolic Disorders**, Vol. 10. CNS Regulation of Carbohydrate Metabolism. ANDREW J. SZABO (ed.). Academic: 1983. Pp. 508. ISBN 0-12-027310-1. \$65.00.
- Advances in Virus Research**, Vol. 28. MAX A. LAUFFER and KARL MARAMOROSCH (eds). Academic: 1983. Pp. 478. ISBN 0-12-039828-1. \$59.
- The Alkaloids: Chemistry and Pharmacology**, Vol. 22. ARNOLD BROSSI (ed.). Academic: 1983. Pp. 342. ISBN 0-12-469522-1. \$50.00.

- Amino Acids: Biosynthesis and Genetic Regulation**. KLAUS M. HERRMANN and RONALD L. SOMERVILLE (eds). Addison-Wesley: 1983. Pp. 453. ISBN 0-201-10520-9. \$41.95.
- Anatomy and Physiology**, 2nd Edn. By EDWIN B. STEEN and ASHLEY MONTAGU. Harper & Row: 1984. Pp. 406. Pbk ISBN 0-06-460190-0. Pbk £4.95.
- Ants**. By PAT and HELEN CLAY. Adam & Charles Black: 1984. Pp. 25. ISBN 0-7136-2386-1. £3.50.
- The Bacterial Cell Surface**. By STEPHEN M. HAMMOND, PETER A. LAMBERT and ANDREW N. RYCROFT. Croom Helm: 1984. Pp. 226. Hbk ISBN 0-7099-1229-3; pbk ISBN 0-7099-1267-6. Hbk £22.50; pbk £10.95.
- Badgers**. By ERNEST NEAL. Adam & Charles Black: 1984. Pp. 25. ISBN 0-7136-2389-6. £3.50.
- Basic Marine Biology**. By A.A. FINCHAM. Cambridge University Press: 1984. Pp. 157. Hbk ISBN 0-521-26421-9; pbk ISBN 0-521-26966-0. Hbk £20, \$36; pbk £7.95, \$14.95.
- Biological Foundations and Human Nature**. The Katzir-Katchalsky Lecture Series. MIRIAM BALABAN (ed.). Academic: 1983. Pp. 243. ISBN 0-12-076150-8. \$39.50, £28.
- Biological Macromolecules and Assemblies**, Vol. 1: Virus Structures. FRANCES A. JURNAK and ALEXANDER MCPHERSON (eds). Wiley: 1984. Pp. 397. ISBN 0-471-87077-3. £69.25.
- Biological Oceanographic Processes**, 3rd Edn. By TIMOTHY R. PARSONS, MASAYUKI TAKAHASHI and BARRY HARGRAVE. Pergamon: 1984. Pp. 330. ISBN 0-08-030765-5. £10.95, \$19.75.
- Biology of Chrysopidae**. M. CANARD, Y. SEMERIA and T.R. NEW (eds). Dr W. Junk: 1984. Pp. 288. ISBN 90-6193-137-1. Dfl. 150, £38.
- The Biology of Crustacea**, Vol. 7. E. JOHN VERNBERG and WINONA B. VERNBERG (eds). Academic: 1983. Pp. 338. ISBN 0-12-106407-7. \$45.00.
- Biology of Microorganisms**, 4th Edn. By THOMAS D. BROCK, DAVID W. SMITH and MICHAEL T. MADIGAN. Prentice/Hall: 1984. Pp. 847. Pbk ISBN 0-13-078338-2. Pbk £14.95.
- Black Caribs: A Case Study in Biocultural Adaptation**. Current Developments in Anthropological Genetics, Vol. 3. MICHAEL H. CRAWFORD (ed.). Plenum: 1984. Pp. 395. ISBN 0-306-41308-6. £59.50.
- Cardiovascular Review 1984**. By GERALD C. TIMMIS et al. Academic: 1984. Pp. 705. ISBN 0-12-691346-3. \$44.
- Cellular Ageing**. Monographs in Developmental Biology 17. H.W. SAUER (ed.). Karger: 1984. Pp. 278. ISBN 3-8055-3860-X. SwFr. 148, DM 177, \$88.75.
- CMV: Pathogenesis and Prevention of Human Infection**. STANDLEY A. PLOTKIN, SUSAN MICHELSON, JOSEPH S. PAGANO and FRED RAPP (eds). Alan R. Liss: 1984. Pp. 515. ISBN 0-8451-1057-8. £67.00.
- Coastal Waders and Wildfowl in Winter**. P.R. EVANS, J.D. GOSS-CUSTARD and W.G. HALE (eds). Cambridge University Press: 1984. Pp. 331. ISBN 0-521-25928-2. £27.50, \$54.50.
- Competition Models in Population Biology**. By PAUL WALTMAN. Wiley: 1984. Pp. 77. Pbk ISBN 0-89871-188-6. Pbk £8.
- Comprehensive Endocrinology. The Pineal Gland**. RUSSEL J. REITER (ed.). Raven: 1984. Pp. 394. ISBN 0-89004-314-0. \$68.50.
- Current Topics in Eye Research**, Vol. 4. JOSE A. ZADUNAIISKY and HUGH DAVSON (eds). Academic: 1984. Pp. 227. ISBN 0-12-153004-3. \$73.00, £56.50.
- Current Topics in Membranes and Transport**, Vol. 22: The Squid Axon. PETER F. BAKER (ed.). Academic: 1984. Pp. 593. ISBN 0-12-153322-0. \$89, £62.50.
- Cytology of the Central Nervous System**. By DOROTHY L. ROSENTHAL. Karger: 1984. Pp. 206. ISBN 3-8055-3808-1. SwFr. 98, DM 117, \$58.75.
- Development of Nerve Cells and their Connections**. By W.G. HOPKINS and M.C. BROWN. Cambridge University Press: 1984. Pp. 137. ISBN 0-521-25344-6; pbk ISBN 0-521-27725-0. Hbk £16, \$29.95; pbk £6.95, \$13.95.
- Developmental Biology of Cultured Nerve, Muscle, and Glia**. By DAVID SCHUBERT. Wiley: 1984. Pp. 315. ISBN 0-471-86592-3. £49.45.

- Disease Resistance in Plants**, 2nd Edn. By J. E. VANDERPLANK. Academic: 1984. Pp. 194. ISBN 0-12-711442-4. \$34.50, £24.50.
- Ecology and Evolutionary Biology: A Round Table on Research**. GEORGE W. SALT (ed.). University of Chicago Press: 1984. Pp. 130. Pbk ISBN 0-226-73443-9. Pbk £6.75.
- The Ecology of Neotropical Savannas**. By GUILLERMO SARMIENTO. Translated by OTTO SOLBRIG. Harvard University Press: 1984. Pp. 235. ISBN 0-674-22460-4. £19.10.
- Ecology of Saprotrophic Fungi**. By R.C. COOKE and A.D.M. RAYNER. Longman: 1984. Pp. 415. ISBN 0-582-44622-1. £20.
- Ecosystem Homeostasis**. By PREZEMYSLAW TROJAN, Dr. W. Junk: 1984. Pp. 132. Pbk ISBN 90-6193-622-5. Pbk Dfl. 85, \$32, £21.50.
- The Endocrinology of Pregnancy and Parturition**. Current Topics in Experimental Endocrinology, Vol. 4. L. MARTINI and V.H.T. JAMES (eds). Academic: 1983. Pp. 290. ISBN 0-12-153204-6. \$44.50.
- The Enzymes**, 3rd Edn. Vol. 16. Lipid Enzymology. PAUL D. BOYER (ed.). Academic: 1983. Pp. 783. ISBN 0-12-122716-2. \$79.00.
- Essential Reproduction**, 2nd Edn. By MARTIN JOHNSON and BARRY EVERITT. Blackwell: 1984. Pp. 367. ISBN 0-632-01258-7. £10.80.
- Evolution of Domesticated Animals**. I.L. MASON (ed.). Longman: 1984. Pp. 452. ISBN 0-382-46046-8. £35.
- Evolutionary Ecology**. B. SHORROCKS (ed.). Blackwell Scientific: 1984. Pp. 418. ISBN 0-632-01189-0. £25.80.
- Evolutionary Theory: Paths into the Future**. J.W. POLLARD (ed.). Wiley: 1984. Pp. 271. ISBN 0-471-90026-5. £21.50.
- Fetal Endocrinology and Metabolism**. Current Topics in Experimental Endocrinology, Vol. 5. L. MARTINI and V.H.T. JAMES (eds). Academic: 1983. Pp. 363. ISBN 0-12-153205-4. \$48.00.
- Fish Physiology**, Vol. 9. W.S. HOAR, D.J. RANDALL and E.M. DONALDSON (eds). Academic: 1983. Pp. 477. ISBN 0-12-350429-5. \$52.00.
- Fish Physiology**, Vol. 9, Part A. W.S. HOAR, D.J. RANDALL and E.M. DONALDSON (eds). Academic: 1983. Pp. 483. ISBN 0-12-350409-0. \$59.00.
- Free Radicals in Biology**, Vol. VI. WILLIAM A. PRYOR (ed.). Academic: 1984. Pp. 437. ISBN 0-12-566506-7. \$75, £52.50.
- From Neuron to Brain**, 2nd Edn. By STEPHEN W. KUFFLER, JOHN G. NICHOLLS and A. ROBERT MARTIN. Sinauer: 1984. Pp. 651. ISBN 0-87893-443-X. Np.
- Frontiers of Oral Physiology. Steroid Hormones in Saliva**. D.B. FERGUSON (ed.). Karger: 1984. Pp. 162. ISBN 3-8055-3848-0. SwFr. 115, DM 138, \$69.
- A Functional Biology of Free-Living Protozoa**. By JOHANNA LAYBOURN-PARRY. Croom Helm: 1984. Pp. 218. Hbk ISBN 0-7099-1625-6; pbk ISBN 0-7099-1678-7. Hbk £16.95; pbk £8.95.
- Ganglioside Structure, Function, and Biomedical Potential**. Advances in Experimental Medicine and Biology, Vol. 174. ROBERT W. LEDEEN, ROBERT K. YU, MAURICE M. RAPPO and KUNIHICO SUZUKI (eds). Plenum: 1984. Pp. 649. ISBN 0-306-41707-3. Np.
- Gastrointestinal Disorders of the Elderly**. By LAWRENCE J. BRANDT. Raven Press: 1984. Pp. 636. ISBN 0-89004-987-4. \$80.50.
- Genetic Analysis of the Cell Surface. Receptors and Recognition**, Series B, Vol. 16. P. GOODFELLOW (ed.). Chapman & Hall: 1984. Pp. 193. ISBN 0-412-25070-5. £27.50.
- Genetics and Development**. By JAMES H. SANG. Longman: 1984. Pp. 398. Pbk ISBN 0-582-44681-3. Pbk £13.95.
- Growth and Maturation Factors**, Vol. 2. GORDON GUROFF (ed.). Wiley: 1984. Pp. 340. ISBN 0-471-09708-X. £66.
- Handbook of the Birds of India and Pakistan**, 2nd Edn. Vol. 4. Frogmouths to Pittas. By SALIM ALI and S. DILLON RIPLEY. Oxford University Press: 1984. Pp. 267. ISBN 0-19-561551-4. £19.50.
- Hydrobiology of the Mangal**. Developments in Hydrobiology. FRANCIS DOV POR and INKA DOR (eds). Dr W. Junk: 1984. Pp. 264. ISBN 90-6193-771-X. Dfl. 180, £45.75.

Immunologische Abwehr und Krebs. By JÜRGEN MILLECK. Akademie-Verlag, Berlin: 1984. Pp.139. DM 48.

Immunology of Anergy and Systemic Lupus Erythematosus. Progress in Allergy, Vol. 35. R.S. SCHWARTZ (ed.). Karger: 1984. Pp.176. ISBN 3-8055-3824-3. DM 117, \$58.75.

In a Patch of Fireweed: A Biologist's Life in the Field. By BERND HEINRICH. Harvard University Press: 1984. Pp.194. ISBN 0-674-44548-1. £15.70.

Infanticide: Comparative and Evolutionary Perspectives. GLENN HAUSFATER and SARAH BLAFFER HARDY (eds). Walter de Gruyter: 1984. Pp.598. ISBN 0-202-02022-3. \$34.95.

International Review of Cytology, Vol. 81. G.H. BOURNE and J.F. DANIELLI (eds). Academic: 1984. Pp.295. ISBN 0-12-364481-X. \$39.50.

International Review of Cytology, Vol. 89. G.H. BOURNE and J.F. DANIELLI (eds). Academic: 1984. Pp.359. ISBN 0-12-364489-5. \$49.50, £35.00.

Intrahepatic Calculi. Progress in Clinical and Biological Research, Vol. 152. KUNIO OKUDA, FUMIO NAKAYAMA and JOHN WONG (eds). Alan R. Liss: 1984. Pp.352. ISBN 0-8451-5002-2. £60.00.

Introduction to Molecular Immunology, 2nd Edn. By ALFRED NISONOFF. Sinauer: 1984. Pp.236. ISBN 0-87893-595-9. £12.80.

Invertebrate-Microbial Interactions. Joint Symposium of The British Mycological Society and the British Ecological Society held at the University of Exeter, September 1982. J.M. ANDERSON *et al.* (ed.). Cambridge University Press: 1984. Pp. 349. ISBN 0-521-25395-0. £40.00, \$79.50.

Lecture Notes on Coastal and Estuarine Studies, Vol. 6, Ecology of Barnegat Bay, New Jersey. MICHAEL J. KENNISH and RICHARD A. LUTZ (eds). Springer-Verlag: 1984. Pp. 396. Pbk ISBN 3-540-90935-4/0-387-90935-4. Pbk DM 96, \$37.70.

The Leukotrienes: Chemistry and Biology. LAWRENCE W. CHAKRIN and DENIS M. BAILEY (eds). Academic: 1984. Pp.308. ISBN 0-12-166750-2. \$52.

ADVERTISEMENT

Federal Biotechnology Funding Sources

Oskar R. Zaborsky, Ph.D.
Brenda K. Young

Vital information on U.S. government programs for:

- Research Scientists and Engineers
- R&D Administrators
- High-Tech Small Businessmen

This invaluable 262-page guide provides the most up-to-date information on extramural R&D programs.

It identifies and describes:

- 329 programs; within
- 33 federal departments and agencies; and
- 282 key contact individuals.

Federal Biotechnology Funding Sources
ISBN 0-931283-23-X
\$69.95

For further information and ordering:

OMEC Publishing Company
1128 Sixteenth Street, N.W.
Washington, D.C. 20036
(202) 833-4161

Metabolism of Hormonal Steroids in the Neuroendocrine Structures. Sero-n Symposium Publications Vol. 13. F. CELOTTI, F. NAFTOLIN and L. MARTINI (eds). Raven: 1984. Pp. 213. ISBN 0-89004-510-0. \$49.

Methods in Enzymology, Vol. 96 Biomembranes. SIDNEY FLEISCHER and BECCA FLEISCHER (eds). Academic: 1984. Pp. 901. ISBN 0-12-181996-5. \$79.

Methods in Enzymology, Vol. 102. Hormone Action Part G, Calmodulin and Calcium-Binding Proteins. ANTHONY R. MEANS and BERT W. O'MALLEY (eds). Academic: 1983. Pp. 336. ISBN 0-12-182002-5. \$41.50.

Methods for the Study of Marine Benthos, 2nd Edn. N.A. HOLME and A.D. MCINTYRE (eds). Blackwell: 1984. Pp. 387. ISBN 0-632-00894-6. £25.00.

Mice. By RON WILSON. Adam & Charles Black: 1984. Pp. 25. ISBN 0-7136-2388-8. £3.50.

Molecular and Cellular Basis of Visual Acuity. S. ROBERT HILFER and JOEL B. SHEFFIELD (eds). Springer-Verlag: 1984. Pp. 184. ISBN 3-540-90964-8/0-540-90964-8. DM 98, \$38.50.

Molecules and Evolution. By A. CULLINAN. British Museum (Natural History): 1984. Pp. 17. Pbk ISBN 0-565-00858-7. Pbk £1.50.

The Mollusca. Vol. 6 Ecology. W.D. RUSSELL-HUNTER (ed.). Academic: 1983. Pp. 695. ISBN 0-12-751406-6. \$69.

Monoclonal Antibodies and Functional Cell Lines. ROGER H. KENNETT, KATHLEEN B. BECHTOL and THOMAS J. McKEARN (eds). Plenum: 1984. Pp. 426. ISBN 0-306-41567-4. Np.

Muscle and Nonmuscle Motility, Vol. 1. ALFRED STRACHER (ed.). Academic: 1983. Pp. 372. ISBN 0-12-673001-6. \$45.

Muscle and Nonmuscle Motility, Vol. 2. ALFRED STRACHER (ed.). Academic: 1983. Pp. 211. ISBN 0-12-673002-4. \$34.50.

Mutations in Man. GÜNTER OBE (ed.). Springer-Verlag: 1984. Pp.327. ISBN 3-540-13113-2/0-387-13113-2. DM 116, \$45.50.

Myelofibrosis and the Biology of Connective Tissue. Progress in Clinical and Biological Research, Vol. 154. PAUL D. BERK, HUGO CASTRO-MALASPINA and LOUIS R. WASSERMAN (eds). Alan R. Liss: 1984. Pp. 520. ISBN 0-8451-50004-9. £52.00.

Neurobiology of the Trace Amines. Analytical, Physiological, Pharmacological, Behavioural and Clinical Aspects. ALAN A. BOULTON *et al.* (eds). Humana Press: 1984. Pp. 624. ISBN 0-89603-063-6. \$59.50.

Neurocommunications: An Introduction. By I.C. WHITFIELD. Wiley: 1984. Pp. 304. ISBN 0-471-10320-9. £17.

Neuroendocrine Aspects of Reproduction. REID L. NORMAN (ed.). Academic: 1984. Pp. 430. ISBN 0-12-521480-4. \$46.

Neuroendocrinology and Psychiatric Disorder. GREGORY M. BROWN, STEPHEN H. KOSLOW and SEYMOUR REICHLIN (eds). Raven: 1984. Pp. 448. ISBN 0-89004-742-1. \$73.50.

Neuroimmunology. Sero-n Symposium Publications Vol. 13. PETER O. BEHAN and FEDERICO SPREAFICO (eds). Raven: 1984. Pp. 500. ISBN 0-89004-963-7. \$105.50.

New Manual of Bryology, Vol. 1. RUDOLF M. SCHUSTER (ed.). Hattori Botanical Laboratory, Miyazaki-ken 889-25, Japan: 1984. Pp. 626. ISBN 4-938163-3045. Np.

General

Collins Guide to the Grasses, Sedges, Rushes and Ferns of Britain and Northern Europe. By RICHARD FITTER and ALASTAIR FITTER. Collins: 1984. Pp.256. Pbk ISBN 0-00-219136-9. Pbk £5.95.

Continuities and Discontinuities in Development. ROBERT N. EMDE and ROBERT J. HARMON (eds). Plenum: 1984. Pp.418. ISBN 0-306-41563-1. Np.

The Dynamics of Nuclear Proliferation. By STEPHEN M. MEYER. University of Chicago Press: 1984. Pp.229. ISBN 0-226-52148-6. £17.

Economic Perspectives on Acid Deposition Control. Acid Precipitation Series Vol.8. THOMAS D. CROCKER (ed.). Butterworth: 1984. Pp.180. ISBN 0-250-40573-3. Np.

European Mires. PETER MOORE (ed.). Academic: 1983. Pp.384. ISBN 0-12-505580-3. £49; \$75.

Evaluation of Research and Development. Proceedings of the Seminar held in Brussels, Belgium, October 17-18, 1983. G. BOGGIO and E. SPACHIS-PAPAZOIS (eds). Reidel: 1984. Pp.147. ISBN 90-277-1759-1. \$27.

The Food Scandal. By CAROLINE WALKER and GEOFFREY CANNON. Century: 1984. Pp.190. ISBN 0-7126-0906-7. £7.50.

A Generous Confidence. Thomas Story Kirkbride and the Art of Asylum-Keeping, 1840-1883. By NANCY TOMES. Cambridge University Press: 1984. Pp.387. ISBN 0-521-24172-3. £27.50, \$39.50.

Health Information Systems: A Bibliography. By DONALD G. ZIEGENFUSS and JAMES T. ZIEGENFUSS. Plenum: 1984. Pp.247. ISBN 0-306-65208-0. \$85.

Human Laterality. By MICHAEL C. CORBALLIS. Academic: 1984. Pp.255. ISBN 0-12-188180-6. \$29.

Introduction to Wastewater Treatment Processes, 2nd Edn. By R.S. RAMALHO. Academic: 1984. Pp.580. ISBN 0-12-576560-6. \$57.

John Muir Summering in the Sierra. ROBERT ENGBERG (ed.). University of Wisconsin Press: 1984. Pp.160. Pbk ISBN 0-299-09624-6. Hbk £20.40; pbk £12.30.

Machines that Think. ISAAC ASIMOV, PATRICIA S. WARRICK and MARTIN H. GREENBERG (eds). Allen Lane: 1984. Pp.626. ISBN 0-7139-1685-0. £10.95.

A Manual of Chemical and Biological Methods for Seawater Analysis. By TIMOTHY R. PARSONS, YOSHIKI MAITA and CAROL M. LALLI. Pergamon: 1984. Pp.173. Hbk ISBN 0-08-030288-2; pbk ISBN 0-08-030287-4. Pbk £5.50, \$8.95.

Marketing Health Behavior: Principles, Techniques and Applications. LEE W. FREDERIKSEN, LAURA J. SOLOMON and KATHLEEN A. BREHONY (eds). Plenum: 1984. Pp.200. ISBN 0-306-41523-2. \$24.50.

Matrix and Finite Element Displacement Analysis of Structures. By D.J. DAWE. Oxford University Press: 1984. Pp.565. ISBN 0-19-856211-X. £35.

Micromorphology of Soils. By E.A. FITZPATRICK. Chapman & Hall: 1984. Pp.433. ISBN 0-412-24200-1. £32.50.

Minimum Steric Difference: The MTD Method for QSAR Studies. By Z. SIMON *et al.* Wiley: 1984. Pp.173. ISBN 0-86380-015-7. £24.90.

The Nature of the Beast: Are Animals Moral? By STEPHEN R.L. CLARK. Oxford University Press: 1984. Pp.127. ISBN 0-19-283041-4. Pbk £2.95.

Nonconventional Energy. G. FURLAN *et al.* (eds). Plenum: 1984. Pp.796. ISBN 0-306-41466-X. \$75.

Otto Hahn: Ein Forscherleben unserer Zeit. By WALTHER GERLACH and DIETRICH HAHN. Wissenschaftliche Verlagsgesellschaft mbH, Postfach 40, D-7000 Stuttgart 01-7: 1984. Pp.267. ISBN 3-8047-0757-2. DM 39.

Review of Progress in Quantitative Nondestructive Evaluation. DONALD O. THOMPSON and DALE E. CHIMENTI (eds). Plenum: 1984. Pp.682. ISBN 0-306-41678-6. Np.

Septic-Tank Systems: A Consultant's Toolkit, Vols 1 & 2. By JOHN H. TIMOTHY WINNEBERGER. Butterworth: 1984. Pp.222, 123. ISBN 0-250-40586-5, 0-250-40634-9. Hbk £30; pbk £19.50.

Spatial Hearing. By JENS BLAUERT. MIT: 1984. Pp.427. ISBN 0-262-02190-0. £36.

Statistical Inference. By VIJAY K. ROHATGI. Wiley: 1984. Pp.940. ISBN 0-471-87126-5. £44.50.

Stellar Nucleosynthesis. CESARE CHIOSI and ALVIO RENZINI (eds). D. Reidel: 1984. Pp.397. ISBN 90-277-1729-X. \$55.

Subrecursion: Functions and Hierarchies. By H.E. ROSE. Oxford University Press: 1984. Pp.189. ISBN 0-19-853189-3. £22.50.

The Śulbasūtras. By S.N. SEN and A.K. BAG. Indian National Science Academy, New Delhi: 1983. Pp.293. \$30.

Sun Calender. By UNA JACOBS. Adam & Charles Black: 1984. Pp.37. ISBN 0-7136-2411-6. £4.50.

Teaching a Stone to Talk: Expeditions and Encounters. By ANNIE DILLARD. Picador: 1984. Pp.177. ISBN 0-330-28341-3. Pbk £2.50.

Test Tube Babies — A Christian View. By IAN DONALD *et al.* Order of Christian Unity: 1984. Pp.98. Pbk ISBN 0-7289-0019-X. Pbk £2.95.

New for immunology and assay

This week's feature covers new immunological products, reagents, sera, monoclonal antibodies and ELISA kits.

● A highly purified peroxidase especially developed as a marker enzyme for enzyme immunoassays is now available from Boehringer Mannheim. The salt-free lyophilizate has a specific activity of at least 250 U per mg (25°C) and a purity number higher than 3.0. The preparation may be used directly for the conjugation without pretreatment.

Circle No. 100 on Reader Service Card.

● The Biomedical Technologies, Inc. epidermal growth factor (EGF) radio-receptor assay kit is capable of measuring the levels of EGF, and also measures other EGF-like peptides from a variety of biological fluids. The term EGF-like peptides includes the peptides termed collectively transforming growth factor- α (TGF- α). The TGF- α proteins, including some proteins produced by transformed cell lines, compete equally for the EGF receptor. The assay measures EGF/TGF- α from a variety of species including mouse and human. Assay sensitivity is 20 picograms per tube.

Circle No. 101 on Reader Service Card.

● Sanbio b.v. of the Netherlands has introduced a large range of monoclonal antibodies for indirect immunoperoxidase staining on tissues and enzyme-linked immunosorbent assay (ELISA) techniques. The series includes anti-human keratin, neurofilament, human chorionic gonadotropin, follicle stimulating hormone and glia fibrillary acidic protein, anti-human vimentin, thyroglobulin and blood vessel endothelium. Sanbio also produces a range of monoclonal antibodies: anti-human T cells and an anti-rat tubulin which will stain eukaryotic cells grown in cell culture as well as frozen sections of tissues. In the United States, Sanbio products are distributed by Accurate Chemical and Scientific Corporation.

Circle No. 102 on Reader Service Card.

● Tissue polypeptide antigen (TPA) is a protein associated with cytoskeletal intermediate filaments of internal epithelial (and hence carcinoma) cells. Rabbit anti-TPA antibody, supplied as 10ml of pre-diluted liquid, can be used for the specific staining of tumour cells in tissue sections using immuno-enzyme techniques. Monitoring serum TPA released by developing tumours using the Prolifigen RIA kit from Sangtec Medical is of use in the control of treatment and detection of recurrence in several types of cancer including breast and intestinal carcinomas. The kit is sufficient for 100 assay tubes. Products are available through Sangtec Medical in Stockholm and through distributors.

Circle No. 103 on Reader Service Card.

● What is claimed to be the first commercially available monoclonal antibody against choline acetyltransferase has been launched by Boehringer Mannheim. This antibody should be of considerable value in the study of the role of cholinergic neurones in the function of the central nervous system. The neurotransmitter synthesizing enzyme choline acetyltransferase is the only reliable marker for cholinergic cells. Immunological demonstration of this enzyme was possible only when highly purified choline acetyltransferase was available as an antigen in order to produce this monoclonal antibody.

Circle No. 104 on Reader Service Card.

● The factor VIII related antigen immunoperoxidase staining kit produced by Biomeda uses avidin-biotin immunotechnology. This immunohistochemical kit is designed to recognize the presence of this blood vessel-endothelial cell marker in paraffin-embedded and frozen tissue sections. The Histoscan-FX VIII kit yields a highly contrasting red colour in positively stained sections. The morphological characteristic of the tissue under study is revealed by the haematoxylin stain which is also part of the kit. The staining methodology using the avidin-biotin affinity cytochemistry (ABAC) gives outstanding staining of tissue sections, and allows very short but efficient incubation steps. The kit is available in two size configurations containing all basic reagents needed to stain either 50 or 150 tissue sections up to the mounting stage. The reagents forming these kits are supplied in a ready-to-use form and are contained in convenient dropper bottles which, for simplicity of use, are both colour and numerically coded.

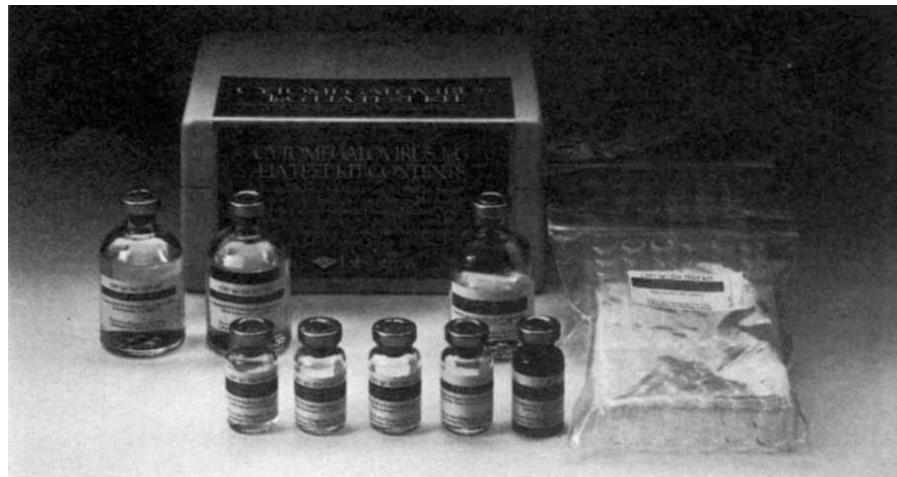
Circle No. 105 on Reader Service Card.

● A new modular system has been introduced by A/S Nunc of Denmark for ELISA and other analyses. The MicroWell Module consists of a frame, into which different types of modules can be fixed to meet individual requirements. The module system is composed of 1×8 and 2×8 well modules with flat bottoms and 2×8 well modules with round bottoms. All three versions are available with high and with medium binding capacity. The frames and the wells of the modules have the same dimensions as a traditional 96-well plate and can thus be used in existing readers, dispensers, washers and so on. The well bottoms are protected against scratches, and selfstanding modules. The same frame is applicable for all the different types of modules. MicroWell Modules are supplied either as loose modules, empty frames or as modules mounted in frames.

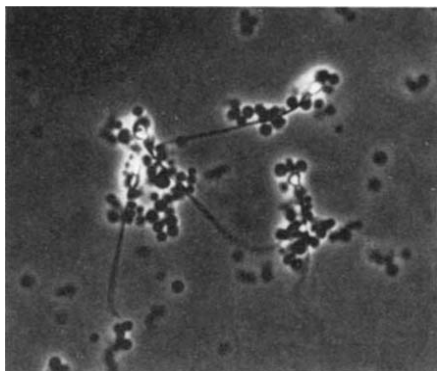
Circle No. 106 on Reader Service Card.

● Antisera to human thyroglobulin and thyroid binding globulin (TBG) are the latest additions to Miles Scientific's line of undiluted hormone/protein antisera. These products are useful in a variety of procedures including gel diffusion, immunohistology and RIA. Anti-human thyroglobulin is a whole serum product enriched by the addition of a concentrated IgG fraction of the same antiserum. The antiserum is produced in sheep, using an antigen prepared from thyroid. Using the peroxidase-anti-peroxidase (PAP) technique, this antiserum has titres of approximately 1:100 against normal thyroid and 1:500 against secondary tumours following thyroidectomy. Anti-human thyroid binding globulin is also an IgG fraction-enriched antiserum. The antigen used is purified from human plasma.

Circle No. 107 on Reader Service Card.



One of the range of ELISA-based kits from Labsystems, this one designed for the diagnosis of cytomegalovirus infection. Circle number 110 for details.



Detection of anti-sperm antibodies with Immunobead from Bio-Rad.

● A rosette-type assay using Bio-Rad's Immunobead reagent allows rapid and reliable detection of anti-sperm antibodies. The assay, performed in 30 minutes, requires only basic laboratory equipment and commercially available reagents. The test can be used either for detection of autoantibodies which are already bound to sperm in semen, or for detection of free anti-sperm antibodies in fluids such as seminal plasma, serum, follicular fluid, or cervical mucus.

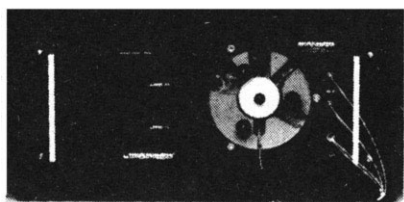
Circle No. 108 on Reader Service Card.

● The Hamilton Microlab M liquid handling system features versatile program storage in a battery buffered memory, making it especially suitable for RIA work. Microlab 1000 is a dual syringe unit, ideal when high dilution ratios are required, and capable of repeat dispense and double dilutions. The most recent addition to the Hamilton range is the Microlab MT, for use with microtitre plates.

Circle No. 109 on Reader Service Card.

ADVERTISEMENTS

Liposomes



LIPOPREP® the equipment for the reproducible preparation of stable unilamellar liposomes of defined size in the range of 30-300 nm with extremely narrow particle size distribution for e.g. the efficient incorporation of (therapeutic) agents incl. antibodies.

DIANORM-Geräte, P.O. Box 126, D-8 München 65, FRG

FM TELEMETRY TRANSMITTER

Smallest & Lightest Available (.5 Grams)
Transmits ECG, EEG, Pressure,
Temperature, Digital Data, Audio, Etc.!



Price—\$449

Write for Fact Sheet

BIOTELEMETRICS, INC.

24650 CENTER RIDGE ROAD #135
WESTLAKE, OHIO 44145 • (216)835-5770

● Five test kits, based on the ELISA technique, are available from Labsystems (UK) Limited. Tetanus IgG EIA kits provide for practical and convenient screening of tetanus antitoxin. The normal sensitivity is 0.1 mIU ml⁻¹ but, if necessary, a more rapid less sensitive test may be performed by decreasing the incubation time to one hour. The CMV IgG EIA test kit allows accurate diagnosis of cytomegalovirus infections. The assay speeds up CMV antibody testing considerably, especially in large screening tests. A positive IgG test suggests previous infection. All the kits are based on solid phase enzyme immunoassay, automatic photometric reading and simple laboratory work. Each kit is complete with all the necessary reagents, sensitized Microstrips or cuvette blocks for the tests.

Circle No. 110 on Reader Service Card.

● A new cell marker, DiI-Ac-LDL (1,1'-di-octadecyl-1-3, 3', 3'-tetramethyl-indo-carbocyanine perchlorate acetylated low density lipoprotein) labels both vascular endothelial cells and macrophages. It can be used to identify and/or isolate these cells from mixed cell populations. When cells are labelled with DiI-Ac-LDL, the lipoprotein is degraded by lysosomal enzymes and the DiI (fluorescent probe) accumulates in the intracellular membranes; there is no effect on cell viability. DiI-Ac-LDL is available from Biomedical Technologies Inc. of Cambridge, Massachusetts.

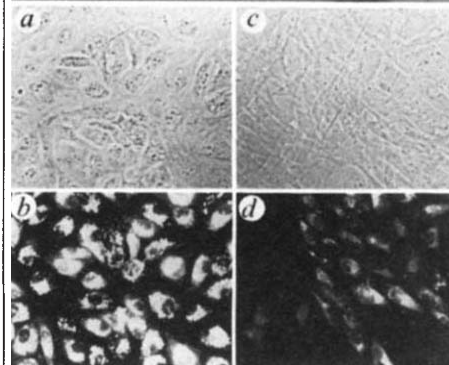
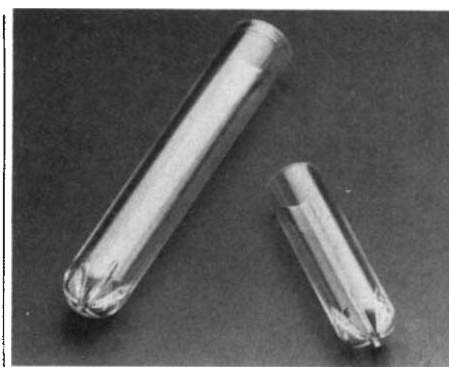
Circle No. 111 on Reader Service Card.

● Leukotriene B₄ can now be measured accurately and conveniently in biological samples using a radioimmunoassay reagents system from Amersham International. The new product consists of ³H-leukotriene B₄ tracer, high specificity antiserum and leukotriene B₄ standard. The measurement range is 12.5 to 400 pg per tube and sufficient reagents are supplied for 125 assay tubes. This permits the construction of a standard curve and the measurement of 53 unknowns in duplicate, but as unused reagents can be stored, a standard curve may be constructed on more than one occasion.

Circle No. 112 on Reader Service Card.

● The ImmunoReader NJ-2000 from Tissue Culture Services is a new approach to automated quantitative photometry. All operations are controlled by an advanced microprocessor which makes it possible to combine a number of standard modes with various parameters as requested by the user. As the NJ-2000 can read up to 15 microplates in sequence, it is suitable for clinical applications and permits a high volume of tests to be performed. Using a keyboard combined with digital displays, the NJ-2000 can be operated without specialized training and user-specific programs can be designed to suit individual analytical needs. Once created, the built-in memory in the NJ-2000 allows eleven programs to be stored for later retrieval when needed.

Circle No. 113 on Reader Service Card.



Top: Nunc's StarTube for ELISA and RIA.

Bottom: Micrographs of cells labelled with the new cell marker from Biomedical Technologies.

a, Phase and b, fluorescence micrographs of a pure culture of bovine aortic endothelial cells. c, Phase and d, fluorescence micrographs of bovine aortic smooth muscle cells and endothelial cells.

● The Nunc StarTube is a specialist tube for ELISA and RIA analyses. It is a round-bottomed polystyrene tube with six wings — a star — on the inside of the tube bottom. The purpose of the star is to increase the surface and thereby make it possible to adsorb greater amounts, more rapidly than is the case with traditional tubes. We have succeeded in increasing the surface by 50-100% dependent on the amount of liquid. The StarTube is manufactured from the same type of polystyrene as the Nunc-Immuno Plate 1, and it can thus adsorb 400 ng immunoglobulins per cm² compared with a tube manufactured from ordinary polystyrene, which will only adsorb 100 ng per cm². The special design of the tube bottom makes it possible to reduce the time required for coating and incubation by about two-thirds without influencing the sensitivity of the analysis. The StarTube is available in two versions: 40 × 10.5 mm and 75 × 12 mm.

Circle No. 114 on Reader Service Card.

● Cellular Products, Inc. of Buffalo, New York and Cordis Laboratories, Inc., of Miami, Florida, have entered into an agreement to jointly produce and market test kits for the detection of antibodies to human T-lymphotrophic viruses (HTLV). Kits are available for both HTLV-I and the AIDS virus (HTLV-III). The system provides quantitative results in less than 3 hours at an approximate cost of \$2.00 per determination.

Circle No. 115 on Reader Service Card.

● A new method for measuring levels of the immunosuppressant cyclosporin has been developed by Water Associates. The HPLC assay monitors cyclosporin in patients without cross-reactivity with its metabolites. Unlike RIA kits, the 15-min assay separates cyclosporin from its metabolites to allow better patient management. Samples are prepared in 30 min using a simple extraction procedure.

Circle No. 116 on Reader Service Card.

● The new electron microscopy grade colloidal gold probes from Janssen are virtually cluster-free immunogold particles with a narrow size distribution. EM-grade colloidal gold is available in four non-overlapping sizes, coupled to secondary antibodies of various specificities, or protein A. Light microscopy (LM) grade immunogold can be used in conjunction with epipolarization (anti-mouse secondary antibody only) or with the ImmunoGold silver staining technique. An immunoblotting (BL) grade range has been developed for the immunochemical visualization of antigen-antibody reactions at the surface of blotting membranes.

Circle No. 117 on Reader Service Card.

● For use with glucose oxidase in immunohistochemical staining processes, Vector Labs has introduced several glucose oxidase substrate kits as companion reagents to Vecta stain ABC-GO immunohistochemical staining kits. Each substrate kit contains optimal concentrations of reagents in convenient dropper bottles and includes a mixing bottle for preparing the substrate solution. The colour of the reaction product depends on the tetrazolium salt supplied with the kit. Three kits are available with a choice of reaction product colours — black, red or purple-blue. The substrate kits include easy to follow instructions and sufficient stock solutions to prepare approximately 300 ml of substrate solution.

Circle No. 118 on Reader Service Card.

● Affinity purified anti-pig IgG (γ) and IgM (μ) are available from Kirkegaard and Perry of Gaithersburg, Maryland. These heavy chain specific polyclonal antibodies were isolated from goat antisera immunized with normal pig IgG or IgM and then affinity purified by exhaustive adsorption on pig IgM and IgG respectively to insure minimal cross-reactivity and high purity. Both antibodies are especially suited to high performance immunoassays that differentiate current and past infections by distinguishing pig IgG or IgM activity. These antibodies are available unlabelled and as peroxidase, phosphatase or fluorescein conjugates.

Circle No. 119 on Reader Service Card.

● Nycodenz Monocytes is a new medium from Nyegaard designed for the one-step isolation of monocytes from leukocyte rich plasma and whole blood. The method is claimed to yield monocytes of high purity (over 90%), good yield (over 60%) and good viability.

Circle No. 120 on Reader Service Card.

● The new 120-page catalogue from Miles Scientific includes an extensive range of immunochemicals. The other products listed are divided into separate sections including: Lectins, Blood Proteins, Polypeptides, Enzymes and Companion Reagents. The catalogue also includes description of LUX tissue culture labware and Lab-Tek general labware.

Circle No. 121 on Reader Service Card.

● The Tago range of antisera now includes goat antisera to human proteins; fluorescein conjugated IgG fractions; antibodies to mouse immunoglobulins; goat anti-rat IgG and goat F(ab')₂ fractions from pepsin-digested affinity isolated antibodies. Both affinity adsorption and affinity isolation are used to purify the Tago reagents.

Circle No. 122 on Reader Service Card.

● A wide range of Bio-Yeda biological products, including many immunochemicals, is now available in the United Kingdom through P & S Biochemicals of Liverpool. Bio-Yeda is a subsidiary of Yeda Research and Development Company of the Weizman Institute of Science in Israel. Amongst the variety of immunochemicals available are: monoclonal antibodies to human immunoglobulins, lactate dehydrogenase isozymes and T and B lymphocytes; monoclonals to DNP, mouse lymphocyte markers and many hormones and drugs; fluorescent and alkaline phosphatase conjugated monoclonals, and monoclonal peroxidase anti-peroxidase complexes. Antisera to almost 100 different human, animal and general cytoskeletal proteins are available. Other Bio-Yeda immunochemicals available include RID plates, IgG fractions of antisera, affinity purified antibodies, RIA/EIA reagents (plus RIA second antibodies) and protein A reagents.

Circle No. 123 on Reader Service Card.

● T-cell growth factor (TCGF, interleukin 2) has been added to Metachem's range of growth and attachment factors. The TCGF is from human leukocytes and is available in 5 ml quantities in sterile, lectin-free liquid containing a small amount of calf serum. The growth factor is stable for one year when stored at 4°C or for 3 days at room temperature.

Circle No. 124 on Reader Service Card.

● The new Imagen RSV test from Boots-Celltech Diagnostics provides an accurate diagnosis of respiratory syncytial virus infection within 30 min. The Imagen RSV test is a rapid, simple direct immunofluorescence test utilizing monoclonal antibodies specific to RSV. This is the second product in the Imagen range to be made available, the first being the Imagen Chlamydia test which was launched earlier this year.

Circle No. 125 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● Hybridoma reagents designed for the easy purification of monoclonal antibodies, titred interleukin 2 (in three purity levels), T-cell reagents and protein-A reagents are now available from Inter-Cell Technologies, Inc. of Somerville, New Jersey. Also available from Inter-Cell are antisera and IgG fractions to human and animal proteins and cells, a wide range of complement reagents, adsorbed antisera with improved specificity to individual cell populations and lymphocytes useful as starting materials for lymphokine production or the isolation of lymphocyte subsets. In addition, Inter-Cell includes among its services custom immunological assays and antisera production.

Circle No. 126 on Reader Service Card.

● A new reagent for immunological research *in vitro* and in laboratory animals removes viability, infectivity and oncogenicity of the viruses (through photochemical inactivation of nucleic acid functions) to provide a new degree of experimental freedom. The reagents, from Lee Biomolecular, are provided in sterile lyophilized form having good shelf life, eliminating the need to propagate, inactivate and standardize viruses in the user's laboratory. More than seventy human and veterinary viruses are listed in the catalogue including herpes simplex, cytomegalovirus, Epstein-Barr and simian virus 40.

Circle No. 127 on Reader Service Card.

ADVERTISEMENT

Copies of articles from this publication are now available from the UMI Article Clearinghouse.

Yes! I would like to know more about UMI Article Clearinghouse. I am interested in electronic ordering through the following system(s):

☐ DIALOG/Dialorder ☐ ITT Dialcom
☐ OnTyme ☐ OCLC ILL Subsystem

☐ Other (please specify) _____

☐ I am interested in sending my order by mail.

☐ Please send me your current catalog and user instructions for the system(s) I checked above.

Name _____

Title _____

Institution/Company _____

Department _____

Address _____

City _____ State _____ Zip _____

Phone (____) _____

UMI Article Clearinghouse

Mail to: University Microfilms International
300 North Zeeb Road, Box 91 Ann Arbor, MI 48106

Meetings

17-18 December, **Meeting on Metal Ions, Free Radical Reactions and Disease**, London (Mr B. Halliwell, Department of Biochemistry, King's College, Strand, London WC2R 2LS, UK).

25 February-1 March, **36th Pittsburg Conference and Exposition on Analytical Chemistry and Applied Spectroscopy**, New Orleans, USA (Mrs Linda Briggs, Program Secretary, 437 Donald Road, Pittsburgh, Pennsylvania, 15235, USA).

26 February-8 March, **Winter Session on Disordered Systems and Biological Organization**, Les Houches, France (Mr G. Weisbuch, Centre de Physique, Univ. Scientifique et Médicale de Grenoble, Côte des Chavants, 74310 Les Houches France).

26-29 March, **3rd European Conference on Food Chemistry**, Antwerp, Belgium (Euro Food Chem. III, Flemish Chemical Society — Food Science Section, Dr P. Dirinck, Faculty of Agricultural Sciences, State University of Gent, Coupure Links 653, B-9000 Gent, Belgium).

27-29 March, **Symposium on the Oceanography of the Rockall Channel** organized by the Royal Society of Edinburgh and the Scottish Marine Biological Association, Edinburgh, UK (The Meeting's Secretary, The Royal Society of Edinburgh, 22, 24 George Street, Edinburgh EH2 2PQ, UK).

31 March-4 April, **Meeting on Process Systems Engineering: SPE '85** — The Use of Computers in Chemical Engineering, Cambridge, UK. The Institution of Chemical Engineers, 165-171 Railway Terrace, Rugby CV21 3HQ, UK).

31 March-5 April, **4th Pfefferkorn Conference on The Science of Biological Specimen Preparation for Microscopy and Microanalysis**, Las Vegas, USA (Dr. Om Johari, PO Box 66507, AMF O'Hare, Illinois 60666 USA).

10-12 April, **Symposium on Recent Advances in Medical and Physiological Imagery**, Harrow Middlesex, UK (Mr M. Goff, Thermographic Laboratory, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK).

15-19 April, **International Symposium on Biomineralization in Lower Plants and Animals**, Birmingham, UK (Dr Robert Riding, Biomineralization Symposium, Department of Geology, University College, Cardiff CF1 1XL, UK).

16-18 April, **International Meeting on Pregnancy Proteins in Animals**, Copenhagen, Denmark (Ms Ulla Salado-Jimena, Department of Pathology, Laboratory Animal Unit, Royal Veterinary and Agricultural University of Copenhagen, Bülowsvej 13, DK-1870 Copenhagen V, Denmark).

22-26 April, **International Symposium-Workshop on Particulate and Multi-Phase Processes, & 16th Annual Meeting of the Fine Particle Society**, Miami Beach, USA (Mr T. Nejat Veziroglu, Director, Clean Energy Research Institute, University of Miami, PO Box 248294, Florida 33124, USA).

24-26 April, **International Symposium on Peptides and Ion Transport**, Florence, Italy (Organizing Secretary, Fondazione Giovanni Lorenzi, Via Monte Napoleone 23, 20121 Milan, Italy).

9-15 June, **21st Chemical Engineering Exhibition**, Frankfurt, FRG (Ms A. Bondaray, V.I.P. Business & Trade Fairs Travel Ltd, 42 North Audley Street, Grosvenor Square, London W1A 4PY, UK).

18-20 June, **Conference on Forest Decline**, Goslar, FRG (Verein Deutscher Ingenieure, Kommission Reinhaltan der Luft, POB 1139 D-4000 Düsseldorf 1, FRG).

The Institute of Physics, London, is holding a series of one-day meetings in London this autumn: 17 October, **Electronics Update**; 23 October, **Applications of Non-linear Analysis**, and **Resonator Sensor Systems**; 24 October, **Critical Phenomena in Magnetism and Low Temperature Physics**; 29 October, **Fundamental Aspects of Polymer Flammability**; 6 November, **Magnetic Thin Film Coatings for the Recording Media**; 8 November, **Low Temperature Techniques Course**; 14 November, **High Complexity Displays**; 21 November, **Drying and Curing**; 5 December, **Electrostatic Imaging**; 12 December, **Transient Annealing of Semiconductors**; 14 December, **Permanent Magnetic Materials and Applications**. Further information from The Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK.

The Scientists Center for Animal Welfare USA, are sponsoring three workshops on **Animals and the Scientist: Institutional Responsibilities**, and **"How to Run an Effective Animal Care and Use Committee**, in conjunction with the following universities: 11-12 October, Michigan State University, Lansing Michigan; 14-15 November, University of Southern California, Los Angeles; and 23-24 May 1985, Aspen Institute, Aspen, Colorado (Dr Barbara Orlans, Scientists Center for Animal Welfare, PO Box 9581, Washington D.C. 20016, USA).

Short courses

19-22 November, **Training Course in Fluid Rheology** (Mr L.J. Viney, Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2BX, UK).

10-12 December, **Yield of Cereals** (Warwick University, UK); 12-14 December, **Yield of Potatoes** (Warwick University, UK) and 5-7 February, **Yield of Sugar Beet** (Peterborough, UK). Further details from the Arable Unit, National Agricultural Centre, Stoneleigh, Kenilworth, Warwickshire CV8 2LZ, UK).

23 April-29 June 1985, **Understanding Climate** (Dr T.M.L. Wigley, Director, Climatic Research Unit, University of East Anglia, Norwich NR4 7TJ, UK).

6-25 January, **International Training Courses on Gene Transfer in Higher**

Eukaryotes, Sofia, Bulgaria (Professor R.G. Tsanev, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria).

3-7 June 1985, **Second International Summer School on Mathematical Modelling in Physiology and Clinical Medicine**, University of Padua, Italy (Dr E.R. Carson, Research Centre for Measurement and Information in Medicine, The City University, Northampton Square, London EC1, UK). **The Centre for Extension Studies, Loughborough University** is organizing a programme of five modular short courses in 1985, leading to the University Diploma in Occupational Health and Safety Management (Mrs K. Gilbert, Centre for Extension Studies, Loughborough, Leicestershire LE11 3TU, UK).

Miscellaneous

The Royal College of Pathologists is introducing a Final Examination in Clinical Cytogenetics open to medical and non-medical candidates. Further details are available from the Examinations Department of the Royal College of Pathologists, 2, Carlton House Terrace, London SW1Y 5AF, UK.

The International Commission on Zoological Nomenclature is calling for nominations for new members to replace staff who are retiring. Nominations should be sent by the end of December 1984 to The Secretary, International Commission on Zoological Nomenclature, British Museum (Natural History), Cromwell Road, London SW7 5BD, UK.

It was recently announced that the **British Computer Society** had been granted a Royal Charter by Her Majesty the Queen.

The Nobeyama Radio Observatory (NRO) has decided to make its 45m telescope available to foreign scientists from January — May 1985 and is calling for study proposals for its use. Those scientists having a Japanese co-investigator will be preferred although in a limited number of cases applications from those without Japanese co-investigators will also be accepted. The deadline for applications will be at the end of October 1984 and further details can be obtained from K. Akabane, Director, Nobeyama Radio Observatory, Nobeyama, Minamisaku, Nagano 384-13, Japan.

A Trauma Research Center, one of the largest of its kind in the world, has been established at the Hebrew University-Hadassah Medical School, Israel.

The European Powder Metallurgy Federation was formed earlier this year in Clermont Ferrand, Switzerland; further information from Mr Robert Wood, The Metals Society, 1 Carlton House Terrace, London SW1Y 5DB, UK.

Laboratory 85 will now form part of British Laboratory week to be held next year from 17-19 September at Olympia, London, UK.